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Epithelial permeability reflects subclinical effects of contact lens wear

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Abstract

***Aims*—Recently, it was reported by the authors that a single drop fluorophotometric technique for estimating corneal epithelial permeability (P_{dc}) to fluorescein is not sufficiently precise for monitoring permeability changes in individual patients, but may be useful for evaluating mean differences in P_{dc} in population based research. To determine whether this technique provides a more sensitive index of epithelial integrity compared with conventional clinical assessments, the effects of mild corneal trauma on P_{dc}, the slit lamp appearance of the cornea, and corneal thickness (CT) were assessed.**

***Methods*—After baseline slit lamp examinations (SLE) and CT measurements, one randomly chosen eye of each of 32 normal subjects underwent 1 hour of closed eye soft contact lens (CL) wear while the fellow eye served as a control (no CL). After removing the CL, the SLE and CT measurements were repeated. Then, P_{dc} to fluorescein was assessed using a single drop fluorophotometric method refined to enhance feasibility, precision, and accuracy.**

***Results*—The mean (95% confidence interval) difference in natural log (P_{dc}) between 32 pairs of eyes (CL minus no CL) was 0.341 (0.069, 0.613), p = 0.016. By contrast, none of the 32 subjects exhibited corneal epithelial disruption upon SLE with white light following the closed eye period. Also, no substantial differences were apparent in the corneal swelling response between paired eyes, mean ΔCT (95% CI) = −2.31(−7.53, 2.91) µm, p=0.37.**

***Conclusions*—P_{dc} measurements, used in studies of modest sample size, appear capable of detecting average differences in corneal barrier function that remain undetectable by SLE or pachymetry.**

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The corneal epithelium protects the anterior ocular surface by providing a barrier to the passage of potentially harmful substances into the cornea. Clinically, the cornea is assessed by slit lamp examination (SLE), which provides largely qualitative information about epithelial integrity.[1] Unfortunately, the SLE does not provide quantitative information about corneal barrier function, and may not be sensitive enough to detect subtle changes in corneal integrity induced by disease (for example, diabetes) or various clinical interventions (for example, contact lens wear, refractive surgery).

Given the limitations of standard clinical methods for assessing epithelial integrity, several investigators have attempted to quantify epithelial barrier function by measuring the rate at which topically applied fluorescein enters the cornea with a fluorophotometer. Since intact epithelial cell membranes and intercellular tight junctions are resistant to the passage of hydrophilic substances such as fluorescein, the diffusion of dye across the corneal epithelial cell layer may indicate a subtle compromise in epithelial integrity.

Until recently, fluorophotometric assessment of epithelial barrier function used an eyebath to deliver fluorescein to the epithelial surface.[2] Since this method of fluorescein application is difficult for many subjects to tolerate, the clinical applicability of the eyebath procedure is limited. Recognising these clinical limitations, Joshi and his co-workers developed a strategy to assess epithelial permeability by applying a single topical drop of fluorescein and determining the rate at which the dye moved from the tears into the cornea.[3] Recently, we refined the single drop methodology and assessed the repeatability of the technique for estimating corneal epithelial permeability (P_{dc}) to fluorescein. In that study we found substantial variability in repeated measurements of P_{dc} on individual subjects which indicated that the technique was not sensitive enough for monitoring permeability changes in an individual patient. However, our data suggested that with appropriate sample size planning, the single drop technique might be useful in population based research to study mean differences in permeability between groups of subjects or for paired eye comparisons.[4]

While the single drop fluorophotometric technique is a promising method for quantifying corneal epithelial barrier function in humans, it is not known whether this technique is sensitive enough to detect subtle changes in epithelial integrity since studies thus far have mainly focused on estimating P_{dc} in the normal cornea. If the single drop paradigm is to have clinical applicability, it must be able to detect changes in corneal barrier function which are not observable using more standard assessments (for example, SLE, pachymetry). In the present study, we used a paired eye comparison design, based upon sample size estimates provided by our previous investigation, to evaluate changes in epithelial permeability induced by 1 hour of closed eye contact lens wear.[4] Permeability changes resulting from this mild dose of epithelial trauma are compared with changes in corneal thickness and slit lamp examination. The goal of this paper was to draw population based inferences about the effect of mild corneal trauma on P_{dc} by examining the distribution of differences between a sample of fellow eyes; we compared fellow eyes, not to draw inferences about individual subjects, but rather as a statistical device to reduce variability due to between subject variation in P_{dc}.

Materials and methods

SUBJECTS

Thirty nine subjects, aged 20 to 44 years (mean age 25 years) with no history of ocular disease or contact lens wear were recruited from the University of California, Berkeley campus. Based upon a slit lamp examination of all eligible subjects using white light, we excluded subjects displaying the following: abnormalities of any corneal layer (that is, any amount of epithelial disruption, dense stromal opacities, corneal degeneration, and/or dystrophy); greater than mild tear film debris; papillae or follicles greater than 0.5 mm in diameter in the upper or lower palpebral conjunctiva; or greater than mild hyperaemia of the bulbar or palpebral conjunctiva. Potential subjects taking systemic medications known to affect tear quantity or those currently suffering from seasonal allergies were also excluded. Informed consent was obtained following a full description of the procedures. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley, Committee for Protection of Human Subjects.

FLUOROPHOTOMETRY

We used the Fluorotron Master automated scanning fluorophotometer to perform all scans as previously described.[4–6] Each measurement consisted of a 5–8 second scan along the optical axis of the eye, beginning at the tear film and passing through the cornea into the anterior chamber. The instrument counted photons of excited fluorescent light at each step and generated a single profile of the combined tear film and corneal fluorescence. If quenching and self absorption are neglected, the area under this fluorescence profile is proportional to the fluorescein mass encountered along the scan path as previously described.[3]

PACHYMETRY

Corneal thickness was measured using a modified Haag–Streit optical pachymeter equipped with fixation lights for improved measurement accuracy resulting in standard deviations of approximately plus or minus 4.0 μm for 10 replicate measurements of central corneal thickness.[7] The pachymeter potentiometer was linked to an IBM compatible microcomputer for direct entry of data to the computer memory. This instrument has been more fully described elsewhere.[8]

INDUCTION OF CORNEAL STRESS

We induced minor trauma by exposing the cornea to a short period of hypoxic stress. A +6.00 D Acuvue disposable lens (42% Etafilcon A/58% water) was inserted in one eye of each subject and both eyes were closed for 1 hour. As previously reported, the oxygen permeability (Dk) of this hydrogel material is 18.0×10^{-11} (cm^2/s) (ml O_2/ml × mm Hg),[9 10] and the harmonic mean oxygen transmissibility (Dk/L_{avg}) of the +6.00 D lens is 14.0×10^{-9} (cm/s) (ml O_2/ml × mm Hg).[11] Thus, we estimated the oxygen tension under the contact lens to be approximately 8 mm Hg versus 55 mm Hg in the fellow eye during the 1 hour of eye closure.[12]

PROCEDURES

All 39 participants were awake at least 2 hours before reporting to the laboratory. Four baseline fluorophotometric scans were averaged for each eye to estimate corneal autofluorescence at the excitation and emission wavelengths of fluorescein. Two corneal thickness (CT) measurements, each the average of 10 replicates obtained within approximately 40–60 seconds, were averaged to provide a baseline (prelens) thickness reading for each eye.

Then, based upon a pre-established randomisation list, one eye of each subject was fitted with a +6.00 D Acuvue disposable CL while the fellow eye served as a control (no CL). The subject rested with both eyes closed for 1 hour, whereafter the contact lens was removed and the CT measurement pattern was immediately repeated to provide a postlens thickness reading for each eye. The absolute change in corneal thickness was obtained by subtracting the prelens CT from the postlens CT (ΔCT = postlens CT − prelens CT).

A postlens SLE was then performed by a masked observer using white light and any disruption to the epithelial surface was graded using a modified version of a system previously used to grade corneal staining with fluorescein.[1] This system divides the cornea into five equally sized zones consisting of a circular central zone and four symmetrically placed quadrants bounded by the horizontal axis, the vertical axis, the inner boundary of the central zone, and an outer circular boundary formed by the limbus. Subjects exhibiting 1+ or greater epithelial disruption in the central cornea (zone 0); 2+ or greater epithelial disruption in any one quadrant of the cornea outside of the central zone (zones 1, 2, 3, 4); or 1+ or greater epithelial disruption in any two peripheral quadrants (zones 1, 2, 3, 4) were not used in the study because our goal was to study P_{dc} changes with subclinical trauma. For this reason, four of 39 potential subjects were excluded at this point as prespecified by our protocol. In addition, three of the 39 subjects experienced reflex tearing during the permeability assessment and we were unable to obtain permeability estimates since the average post rinse stromal fluorescence value was less than the average background fluorescence for the same eye. The randomisation assignment for each of these seven subjects was returned to the end of the queue and recruitment continued to obtain the 32 subjects needed to complete the balanced randomised design.

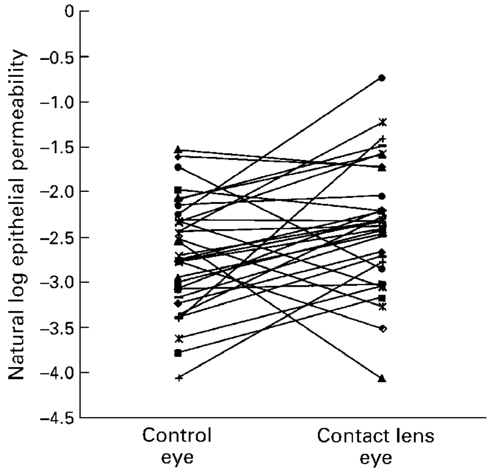

Figure 1 Natural log epithelial permeability following 1 hour of eye closure with a contact lens and without a contact lens (control eye). The two eyes of each subject are plotted with the same symbol and connected by a straight line.

To estimate permeability, a micropipette was to used to deliver 2 μl of 0.35% fluorescein onto the central superior bulbar conjunctiva of the subject's right or left eye, and the subject was instructed to close and roll his/her eye in order to evenly distribute the fluorescein to the tear film. The eye was scanned immediately after dye instillation, and the same procedure was repeated on the fellow eye. The two eyes were scanned alternately every 2 minutes for the next 20 minutes and then thoroughly rinsed three times with non-preserved, sterile saline. Stromal fluorescein levels were measured five times over the next 10 minutes. Permeability estimates were obtained as previously described.[3 4]

STATISTICAL METHODS

Earlier reports suggested transforming P_{dc} by the natural logarithm function before analysis.[3 4 13] Residual plots of our data confirmed that this transformation stabilised the within subject variability of P_{dc} and induced greater symmetry; thus, our analyses of P_{dc} are on the natural logarithm (ln) scale. For symmetric distributions, the median and mean coincide and since median[ln(P_{dc})] = ln[median P_{dc}], an estimate of the mean on the ln(P_{dc}) scale (units = ln(nm/s)) can be back transformed to give a reasonable estimate of median P_{dc} (units = nm/s).

In a previous study, we found systematically greater P_{dc} estimates in left versus right eyes, which could have occurred because of some asymmetry in the instrumental set up and/or because the P_{dc} measurement procedure always began on the subject's right eye.[4] In the present study, the eye receiving the stress lens and the eye to be measured first during the P_{dc} assessment were randomised using a balanced block design with eight consecutive blocks of four subjects each. With this design, an equal number of right and left eyes received the lens and an equal number of right and left eyes began the measurement sequence. Moreover, this design ensured that after every block of four subjects was randomised, the subjects were equally distributed among the four randomly ordered combinations of these two variables: right eye stressed and measured first; right eye stressed and measured last; left eye stressed and measured first; left eye stressed and measured last.

The balanced design enables us to estimate the average treatment effect (lens versus no lens) by simply calculating the average of the paired eye differences in ln(P_{dc}), which are statistically independent. Using these same paired eye differences in ln(P_{dc}) as the outcome variables, we employed standard two way fixed effects analysis of variance (ANOVA) techniques to simultaneously estimate the effects of treatment, right versus left eye, and measurement order on ln(P_{dc}).[14] Finally, to estimate variance components and their standard errors, we fitted mixed effect analysis of variance models[15] to the individual eye ln(P_{dc}) measurements, accounting for potential correlation between fellow eyes through appropriate specification of the covariance structure. The mixed model estimates were produced using standard maximum likelihood techniques implemented in BMDP Program 5 V.[16]

Results

SLIT LAMP EXAMINATION

There were 32 subjects who provided data for analysis. Following 1 hour of eye closure, both the control and experimental eyes of these 32 subjects were free of epithelial disruption upon SLE by a masked observer using white light.

CORNEAL THICKNESS

Following 1 hour of eye closure, the mean absolute change in corneal thickness (ΔCT = postlens CT − prelens CT) was 12.2 μm (95% confidence interval: 9.5, 15.0) in the contact lens wearing eyes and 14.5 μm (95% CI 10.9, 18.2) in the control non-lens wearing eyes. The mean difference in absolute corneal swelling between paired eyes (experimental eye − control eye) was −2.3 μm (95% CI −7.5, 2.9), which is neither clinically nor statistically significantly different from zero (p=0.37). Thus, we found no evidence of an important difference in corneal swelling between the experimental and control eyes.

EPITHELIAL PERMEABILITY

Figure 1 displays the ln(P_{dc}) measurements for each control and experimental eye. The measurements for the two eyes of each subject are connected by a line and are represented by the same symbol. The mean ln(P_{dc}) measured in the lens wearing eye was −2.35 (95% CI −2.61, −2.10) compared with −2.69 (95% CI −2.92, −2.47) in the control eye. Back transforming the mean ln(P_{dc}) yields median P_{dc} estimates for the lens wearing and control eyes of 0.095 nm/s and 0.068 nm/s, respectively.

Figure 2 displays a box and whisker plot of the differences in ln(P_{dc}) between paired experimental and control eyes. The lower and upper bounds of the box represent the 25th and 75th percentiles of the observed distribution of ln(P_{dc}) differences, respectively; the

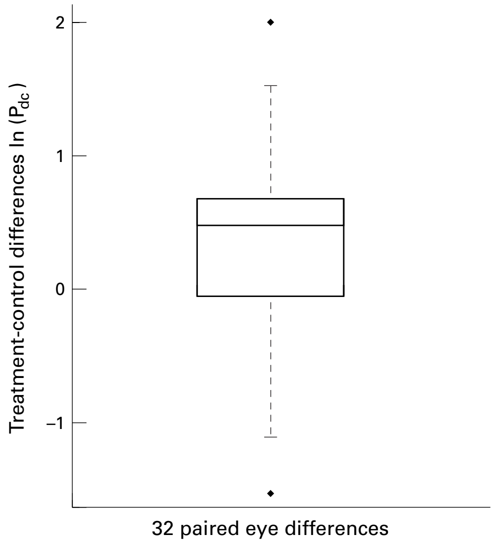

Figure 2 Box and whisker plot of the distribution of differences in natural log epithelial permeability between paired eyes (contact lens v control).

central line represents the median. The vertical lines extend out from the box to the most distant observation within 1.5 times the distance between the 25th and 75th percentiles; outliers are represented by isolated solid diamonds beyond these "whiskers." The mean difference in $\ln(P_{dc})$ between the paired experimental and control eyes, which estimates the average change in P_{dc} induced by 1 hour of closed eye lens wear versus 1 hour of eye closure without a lens, was 0.341 (95% CI 0.069, 0.613) ($p=0.016$; two sided *t* test).

The preceding $\ln(P_{dc})$ comparisons did not explicitly account for whether the right or left eye was the stressed, experimental eye, and which eye was measured first. However, the experimental design, through blocked randomisation, balanced the comparisons with respect to these two factors; thus, these simple averages are "controlled" for potential eye and order effects by virtue of the study design.

Standard two way ANOVA techniques applied to the differences in $\ln(P_{dc})$ between fellow eyes provide a way to simultaneously estimate systematic differences between eyes with and without lenses, right and left eyes, and eyes measured first versus second. Table 1 presents these estimates along with their confidence intervals and p values.

Table 1 The mean differences in $\ln(P_{dc})$ between the two levels of each categorical variable estimated by fitting a two factor fixed effects models to the fellow eye differences in $\ln(P_{dc})$. p Values were estimated using two sided t tests

	Mean difference	*95% CI*	*p Value*
Lens – no lens	0.341	0.063, 0.169	0.02
Right – left	−0.013	−0.265, 0.290	0.93
First – second	−0.127	−0.405, 0.151	0.36

Table 2 Variance components estimated using a mixed effects model and their standard errors

Source of variation	*Variance component estimate*	*Standard error*
Between subject variance		
average $\ln(P_{dc})$	0.1557	0.0805
treatment effect	0.1094	0.1435
Within subject variance	0.2208	0.0895

Since our design was balanced with respect to eye and measurement order, the average difference reported in Table 1 between experimental and control eyes, estimated while controlling statistically for eye and order effects through the ANOVA model, is identical to the simple average of these differences reported above. The average difference in $\ln(P_{dc})$ between right and left eyes is negligible and statistically indistinguishable from zero. The mean difference in $\ln(P_{dc})$ between eyes measured first versus second was on average, $-0.127 \ln(P_{dc})$, and also was not statistically significant.

Fitting a mixed effects model to the $\ln(P_{dc})$ measurements facilitates a comprehensive examination of the components of variance. The model underlying Table 2 attempts to disentangle two potential sources of between subject variation in $\ln(P_{dc})$ measurements: subjects may vary in their average $\ln(P_{dc})$ following 1 hour of eye closure; in addition, subjects may vary in their $\ln(P_{dc})$ response to 1 hour of lens wear (that is, while the average treatment effect is 0.341, the response of individual subjects may vary about this average). The within subject variance component reflects variation within individuals' $\ln(P_{dc})$ measurements not accounted for by the treatment effect. These variance components are important not only for sample size planning, but for choosing an appropriate study design.

Discussion

Among our 32 pairs of study eyes, we observed an average increase of 41% in epithelial permeability in eyes exposed to only 1 hour of contact lens induced hypoxia relative to the fellow contralateral eyes that were closed for 1 hour without contact lenses. Interestingly, this mild corneal trauma typically did not produce a detectable difference between paired eyes in either the slit lamp appearance of the cornea or corneal thickness. These results suggest that the assessment of epithelial barrier function using fluorophotometry may provide a more sensitive index of subtle damage to the ocular surface than obtained using either the slit lamp or pachymeter.

While this study was not designed to address the clinical relevance of a substantial increase in epithelial permeability to fluorescein following 1 hour of closed eye contact lens wear, it does suggest that routine use of hydrogel lenses on an extended wear basis may alter epithelial integrity. Of clinical interest may be that this significant increase in P_{dc} occurred without detectable changes in slit lamp examination or corneal thickness. Many of the most serious corneal complications resulting from contact lens wear occur without prior signs of contact lens induced keratopathy, and it would be useful to determine if we can predict the occurrence of these complications using the P_{dc} measurement. To address this issue, it will be important to conduct a prospective study of epithelial permeability in subjects wearing soft lenses on an extended wear basis in order to determine the association between changes in

P_{dc} and the development of contact lens associated complications.

Previous investigators have been unable to identify contact lens induced alterations to epithelial barrier function using a fluorescein eyebath technique.[17 18] The results of one such study actually measured a decrease in epithelial permeability in contact lens wearers compared with controls and suggested that use of a hydrogel lens may protect the corneal epithelium against the exfoliative effects of blinking and, thereby, increase epithelial barrier function.[18] Furthermore, no significant differences were found in P_{dc} measurements obtained on a group of subjects following specified periods of daily and extended wear of hydrogel lenses, and the authors conclude that contact lens associated infectious keratitis is not due to changes in epithelial barrier function.[17] However, these investigators did not measure P_{dc} in the extended wear eyes immediately upon eye opening (JA van Best, personal communication), and it is possible that permeability is increased immediately following closed eye wear of a hydrogel lens and then recovers to a normal level at some later time after the eye is open. Our results demonstrate that P_{dc} changes in response to relatively minor trauma; therefore, the question remains open as to whether short term increases in corneal epithelial permeability may account for the increased incidence of bacterial infections associated with extended wear.

In a previous study,[4] we observed considerable between subject variability in P_{dc}. We therefore attempted to minimise the impact of between subject variability in the present study by analysing the differences between fellow eyes, which caused any variability operating at the subject level on both eyes to cancel. This appeared to provide a useful design strategy since our sample size of 32 subjects was sufficient to detect a relatively modest difference in P_{dc} between the experimental and control eyes despite the considerable variability associated with the measurement technique. This sample size was close to what we predicted to be necessary to detect a 40% difference in P_{dc} between paired eyes with power of 0.90.[4] We should note, however, that while our previous work suggested that between person variability in P_{dc} was much more substantial than within person variability, Table 2 suggests that in this study, the within subject component may be larger (within subject = 0.2208 versus between subject = 0.1557). In addition, Table 2 suggests that there may be considerable between person variation in the treatment effect which is consistent with clinical observations of different individual responses to the same treatment. The relatively large standard errors in Table 2, however, indicate that our knowledge of these variance components remains imprecise. Thus, while the paired eye comparison was a useful approach here, it may still be beneficial to determine whether it is more statistically efficient to randomise subjects or fellow eyes in studies designed with P_{dc} as the outcome.

Consistent with our previous findings,[4] Figure 2 highlights the difficulty in utilising our procedure for monitoring *individual* patients. Although we had sufficient statistical power to detect an average increase in ln P_{dc} of 0.341 using the *group* of 32 fellow eye comparisons, Figure 2 illustrates that for approximately 25% of our 32 subjects, the observed P_{dc} was, in fact, greater in the control than in the treatment eye. The broad range of these differences (−1.5 to 2.0 ln P_{dc}) reflects the effects of between subject variation in treatment effect along with within subject variation in P_{dc}. Several possible mechanisms could contribute to the observed variability in our P_{dc} estimates. For example, stimulation of reflex tearing when the dye is instilled would leave minimal fluorescein available for corneal uptake. This may result in post rinse fluorescence values that are only slightly greater than those measured at baseline, which is likely to degrade the precision of the P_{dc} measurement. Additional discussion about the potential sources of variability in the P_{dc} measurement is provided in a previous publication.[4]

We also found systematically greater estimates of P_{dc} on left versus right eyes in our previous study and hypothesised that this may have occurred because of some asymmetry in the instrumental set up and/or from the measurement order since right eyes were always assessed first. We found little evidence of a right versus left eye effect. The data also provide no clear evidence of substantial order effect; however, the lower bound of the 95% confidence interval extends to −0.405 ln(P_{dc}), and thus is consistent with a relatively substantial order effect. We, therefore, recommend that experimental protocols involving fellow eyes use randomised measurement order to rule out this potential bias.

This investigation suggests that for group comparisons, P_{dc} estimates obtained using a convenient, single drop fluorophotometric technique provide a more sensitive marker for alteration to the ocular surface than routine slit lamp examination and pachymetry. The aetiology of this increase in permeability is uncertain. The difference in epithelial permeability between paired eyes following 1 hour of closed eye contact lens wear did not appear to be due to obvious alteration to the corneal epithelium since a masked observer was unable to distinguish between the experimental and control eyes following the period of eye closure. In fact, only four out of 39 subjects recruited for this study demonstrated noticeable differences in epithelial integrity in the experimental eye compared with the control eye upon SLE. Since our goal was to study P_{dc} changes in eyes without clinically visible alterations in epithelial integrity, we conducted the SLE immediately before the permeability assessment. The use of fluorescein dye during this procedure would have interfered with the permeability assessment; therefore, our trained observers carefully examined both corneas with white light following the hour of eye closure, with particular attention given to the central zone where epithelial permeability was assessed.

While this method for detecting epithelial alteration may be less sensitive than grading corneal staining with the aid of fluorescein dye, the epithelium becomes readily visible with white light following any trauma sufficient to disrupt optical homogeneity due to an increase in backscatter of light.[19] We also found no evidence of an association between P_{dc} and the minimal amounts of corneal swelling we induced.

In sum, the single drop measurement of epithelial permeability appears to provide a sensitive and useful tool for quantifying the effects of closed eye contact lens wear on the epithelium in population based research. Moreover, use of this relatively simple, non-invasive procedure may help us improve our understanding of the fundamental factors that lead to altered epithelial barrier function.

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