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 (Pdc) to fluorescein is not sufficiently precise for monitoring permeability changes in individual patients, but may be useful for evaluating mean differences in Pdc 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 Pdc, 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, Pdc 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 (Pdc) 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—Pdc 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. (Br J Ophthalmol 1998;82:376–381)

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 quantifiable 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 topical 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 eyewash 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 eyewash 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 (Pdc) to fluorescein. In that study we found substantial variability in repeated measurements of Pdc 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 Pdc 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.5 Permeability changes resulting from this mild dose of epithelial trauma are compared with changes in corneal thickness and slit lamp
Epithelial permeability reflects subclinical effects of contact lens wear

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. 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.

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. 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.

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% Etafiticon 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 $18.0 \times 10^{-11}$ (cm$^2$/s) (ml O$_2$/ml × mm Hg)$^{9,10}$ and the harmonic mean oxygen transmissibility (Dk/Lav) of the +6.00 D lens is $14.0 \times 10^{-6}$ (cm$^2$/s) (ml O$_2$/ml × mm Hg). 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.

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 (ACT = 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. 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 $P_{	ext{ec}}$ 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.
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TRATNET
The ln(Pdc) scale (units = ln(nm/s)) can be back
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the natural logarithm function before
transformed to give a reasonable estimate of
greatersymmetry; thus, our analyses of Pdc are
mean coincide and since median[ln(Pdc)]
symmetric distributions, the median and
on the natural logarithm (ln) scale. For

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 meas-
ured five times over the next 10 minutes.
Permeability estimates were obtained as previ-
ously described.1 4

STATISTICAL METHODS
Earlier reports suggested transforming Pdc by
the natural logarithm function before
analysis.1 4 13 Residual plots of our data con-
firmed that this transformation stabilised
the within subject variability of Pdc and induced
greater symmetry; thus, our analyses of Pdc are
on the natural logarithm (ln) scale. For
symmetric distributions, the median and
mean coincide and since median[ln(Pdc)]
= ln[median Pdc], an estimate of the mean on
the ln(Pdc) scale (units = ln(nm/s)) can be back
transformed to give a reasonable estimate of
median Pdc (units = nm/s).

In a previous study, we found systematically
greater Pdc estimates in left versus right eyes,
which could have occurred because of some
asymmetry in the instrumental set up and/or
because the Pdc measurement procedure always
began on the subject’s right eye.1 4 In the present
study, the eye receiving the stress lens and the
eye to be measured first during the Pdc
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(Pdc), which are sta-
tistically independent. Using these same paired
eye differences in ln(Pdc) as the outcome
variables, we employed standard two way fixed
effects analysis of variance (ANOVA) tech-
niques to simultaneously estimate the effects of
treatment, right versus left eye, and
measurement order on ln(Pdc).1 4 Finally, to
estimate variance components and their stand-
ard errors, we fitted mixed effect analysis of
variance models17 to the individual eye ln(Pdc)
measurements, accounting for potential corre-
lation between fellow eyes through appropriate
specification of the covariance structure. The
mixed model estimates were produced using
standard maximum likelihood techniques im-
plicated in BMDP Program 5 V.18

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 (ACT =
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(Pdc) measurements for
each control and experimental eye. The mea-
surements for the two eyes of each subject are
connected by a line and are represented by the
same symbol. The mean ln(Pdc) 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(Pdc) yields median
Pdc estimates for the lens wearing and control
eyes of 0.095 nm/s and 0.068 nm/s, respecti-
vely.

Figure 2 displays a box and whisker plot of
the differences in ln(Pdc) between paired
experimental and control eyes. The lower and
upper bounds of the box represent the 25th
and 75th percentiles of the observed distribu-
tion of ln(Pdc) differences, respectively; the
Table 2 Variance components estimated using a mixed effects model and their standard errors

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Variance component estimate</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subject variance</td>
<td>0.1557</td>
<td>0.0805</td>
</tr>
<tr>
<td>treatment effect</td>
<td>0.1094</td>
<td>0.1435</td>
</tr>
<tr>
<td>Within subject variance</td>
<td>0.2208</td>
<td>0.0895</td>
</tr>
</tbody>
</table>

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

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 model to the fellow eye differences in $\ln(P_{dc})$. $p$ Values were estimated using two sided t tests

<table>
<thead>
<tr>
<th></th>
<th>Mean difference</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens – no lens</td>
<td>0.341</td>
<td>0.063, 0.169</td>
<td>0.02</td>
</tr>
<tr>
<td>Right – left</td>
<td>−0.013</td>
<td>−0.265, 0.290</td>
<td>0.93</td>
</tr>
<tr>
<td>First – second</td>
<td>−0.127</td>
<td>−0.405, 0.151</td>
<td>0.36</td>
</tr>
</tbody>
</table>

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
Previous investigators have been unable to identify contact lens induced alterations to epithelial barrier function using a fluorescein eye bath technique. 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. Furthermore, no significant differences were found in $P_a$ measurements obtained on a group of subjects following specific 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. However, these investigators did not measure $P_a$ 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 $P_a$ 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, we observed considerable between subject variability in $P_a$. 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 $P_a$ 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 $P_a$ between paired eyes with power of 0.90. We should note, however, that while our previous work suggested that between person variability in $P_a$ 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 $P_a$ as the outcome.

Consistent with our previous findings, 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 $\ln P_a$ of 0.341 using the group of 32 fellow eye comparisons, Figure 2 illustrates that for approximately 25% of our 32 subjects, the observed $P_a$ was, in fact, greater in the control than in the treatment eye. The broad range of these differences (−1.5 to 2.0 $\ln P_a$) reflects the effects of between subject variation in treatment effect along with within subject variation in $P_a$. Several possible mechanisms could contribute to the observed variability in our $P_a$ 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 fluorescein values that are only slightly greater than those measured at baseline, which is likely to degrade the precision of the $P_a$ measurement. Additional discussion about the potential sources of variability in the $P_a$ measurement is provided in a previous publication.

We also found systematically greater estimates of $P_a$ 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 $\ln(P_a)$, 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, $P_a$ 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 $P_a$ 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. We also found no evidence of an association between Pdc 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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