Estimation of Human Corneal Oxygen Consumption by Noninvasive Measurement of Tear Oxygen Tension While Wearing Hydrogel Lenses

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PURPOSE. To devise a procedure for direct estimation of corneal oxygen consumption in human subjects.

METHODS. Tear oxygen tension (PO2) was measured at the posterior surface of two standard hydrogel contact lenses (38% water, 0.2 and 0.06 mm thick, oxygen transmissibility [Dk/t] = 4.2 and 14 × 10−9 cm·mL O2/mL·sec·torr) and one newly available hydrogel-silicone polymer lens (Dk/t = 99 × 10−9 cm·mL O2/mL·sec·torr). The oxygen-sensitive dye, Pd-meso-tetra (4-carboxyphenyl) porphine, bound to bovine serum albumin, was incubated with the lenses overnight. The lenses, coated with the protein-dye complex, were placed on four subjects’ eyes, and tear PO2 was measured in the open eye and after 5 minutes of eye closure, using a time-domain phosphorescence measurement system. Given the tear Po2, lens Dk/t, and corneal thickness, oxygen consumption (Qc, in mL O2/cm3·sec) could be calculated from established oxygen diffusion models.

RESULTS. Protein-dye complex bound to the lens surface enabled reporting of tear PO2 for long periods. As expected, estimated tear PO2 was higher in subjects wearing lenses with higher Dk/t: mean open-eye PO2 = 30.6 ± 3.1 and 8.1 ± 1.3 torr for the thin and thick hydrogel lenses, respectively, and 97.6 ± 22.9 torr for the hydrogel-silicone lens. After 5 minutes of eye closure, tear PO2 was significantly reduced and reached a new steady state in approximately 20 seconds after eye opening. Fitting a single exponential model to the data and extrapolating to t = 0 provided an estimate of PO2 under the closed lid for the thin hydrogel (PO2 = 7 ± 2.5 torr) and the hydrogel-silicone lens (PO2 = 22.6 ± 4 torr). After 5 minutes of eye closure with the thick hydrogel lens, tear PO2 remained constant for ~10 seconds after eye opening (mean PO2 = 3.9 ± 0.7) before increasing to a new steady state. This delay could be accounted for by the time needed for oxygen to diffuse to the posterior surface of the lens. Calculated Qc ranged from 2.2 × 10−4 to 3.7 × 10−6 mL O2/cm3·sec at the highest and lowest PO2s, respectively, and is comparable to previous in vitro and in vivo estimates.

CONCLUSIONS. Tear PO2 behind hydrogel lenses can be measured in human subjects using the phosphorescence of the porphyrin-protein complex bound to the lens surface. The method is simple, fast, reliable, and noninvasive, allowing quick and direct estimates of Qc. In addition to contact lens wear, this method should be useful for examining the effects of disease, surgery, or topical drugs on the corneal oxygen consumption rate. (Invest Ophthalmol Vis Sci. 2002;43:371–376)

Assessment of metabolic activity in vivo by noninvasive techniques is a desirable approach for studying the normal physiology of ocular tissues and how it is altered by disease and drugs, surgery, or other interventions. Fluorescence- and phosphorescence-based techniques offer high sensitivity and are applicable to studying the physiology of many ocular structures that are optically accessible. Autofluorescence of naturally occurring substances (e.g., the reduced nicotinamide adenine dinucleotide-to-nicotinamide adenine dinucleotide [NADH/NAD] ratio) can detect the tissue’s metabolic state and is advantageous, because addition of exogenous agents is not required. Unfortunately, the autofluorescence signal is typically very weak or may require ultraviolet illumination, but two-photon techniques could circumvent the radiation hazard. Fluorescent and phosphorescent dyes are more sensitive, but must be delivered in usable concentrations to the site of interest and/or may have toxic interactions, which could limit clinical applicability.

Previously, we have reported the use of a phosphorescence-quenching technique to measure the tear PO2 beneath contact lenses in rabbits. This method has also been used to determine anterior chamber and retinal vasculature PO2. Although it is of interest to contact lens researchers and manufacturers to use tear PO2 to assess lens performance, this approach can also be used to measure corneal oxygen consumption (Qc) in vivo. Clinical response to contact lens wear and laboratory hypoxia-induced corneal swelling studies have hinted that there is a wide variability in corneal oxygen demand in the normal young population. Furthermore, it has been shown that oxygen uptake into corneas of diabetic rabbits and humans is suppressed. Thus a sensitive, easily administered, and quick measurement of Qc could be useful in studying the effects of disease, surgery, topical drug use, or contact lens wear on the metabolic status of the cornea.

The steady state tear PO2 under a contact lens is determined primarily by Qc. Therefore, given the tear PO2 under a contact lens of known oxygen transmissibility (Dk/t), it is possible to estimate Qc. A previous attempt to directly measure in vivo human Qc required the use of tight-fitting, fluid-filled gogles and oxygen-consuming Clark-type electrodes. A less invasive, but indirect and time-consuming approach, relied on corneal swelling responses to estimate the PO2 in subjects wearing contact lenses of known transmissibility. In contrast, the phosphorescence technique that we report in the current study, is a direct measurement of tear PO2 beneath contact lenses that requires only a few minutes of hydrogel contact lens wear.
METHODS

Subjects

Four subjects (two men, two women; mean age, 25 ± 2 years) who were free of ocular and systemic disease and had not worn contact lenses for at least 6 months participated in this study. The research adhered to the tenets of the Declaration of Helsinki and was approved by the Indiana University Human Subjects Committee.

Instrumentation

The principles of oxygen measurement by phosphorescence quenching have been previously described in detail. Briefly, a 10-μsec excitation flash excites a probe whose phosphorescence is quenched by oxygen. The relationship between phosphorescence decay lifetime and oxygen concentration follows a linear relation described by the Stern-Volmer equation. We used a commercially available system (Oxyspot; Medical Instruments, Inc; now available through Harvard Apparatus, Holliston, MA). The flash excitation was coupled to the illumination optics of a slit lamp (model FS-1; Nikon, Melville, NY) through a fiber optic cable. The slit was adjusted to provide a 2 × 2 mm² illumination. Phosphorescence was collected by a gated photomultiplier tube mounted on the slit lamp's camera port. Gating delay, sample number, and sampling rate were controlled by computer (Oxyspot software; Harvard Apparatus, running on a Windows 95 [Microsoft, Redmond, WA]–compatible computer). The phosphorescence decay constant (τ) was computed after each flash, and the average τ of 10 successive flashes was computed for each P02 data point. Also determined was the correlation coefficient for the fit of the data to a first-order exponential decay. At a P02 less than 50 torr, data with correlation coefficients less than 0.9 were rejected. At higher P02 levels, a correlation coefficient of 0.8 was used as the cutoff. Typically, poor correlations occurred during blinks or eye movements.

Lenses

Three contact lenses were used: (1) a newly available hydrogel-silicone lens with Dk/t = 99 × 10⁻⁹ (Balafilcon; Bausch & Lomb, Rochester, NY; Dk = 99 × 10⁻¹¹ cm² · mL O₂/mL · sec · tor; thickness = 0.1 mm), a 38% water lens with Dk/t = 14 × 10⁻⁹ (Polycon, Dk = 8.4 × 10⁻¹¹, thickness = 0.06 mm; Metroptics, Glendora, CA), and a 0.2 mm thickness lens (Dk/t = 4.2 × 10⁻⁹; Polycon; Metroptics). All lenses had back surface radii of 8.6 mm, diameter of 13.5 mm and −0.50 D power to achieve uniform thickness.

Preparation of Oxygen-Sensitive Dye and Lens

A 1:9 part mixture of the oxygen-sensitive phosphorescent dye Pd meso-tetra-(-carboxyphenyl) porphine and bovine serum albumin (BSA) was obtained from Harvard Apparatus. The powder was dissolved into Ringer’s solution with final concentrations (in mM) of 140 NaCl, 2 K₂HPO₄, 0.61 MgCl₂, 1.4 Ca⁺⁺-gluconate, and 28.5 Na⁺⁺-gluconate (pH 7.5). Osmolarity was adjusted to 300 ± 5 mOsm. The solution was forced through a 0.2-μm filter and placed in a sterile container. A new sterile contact lens was placed in the dye solution and incubated overnight at room temperature. The next day, the lens was rinsed with sterile saline and placed on the subject’s right eye.

It is conceivable that a protein coating on the surface of a contact lens could provide another layer of resistance to the passage of oxygen across the lens. This would potentially decrease the Dk/t of the lens. Indeed, very thick, denatured albumin coatings placed on contact lenses have been found to lower the amount of oxygen reaching the cornea, but coatings of such thickness are not encountered in practice. Protein coatings from normal wear or mild protein applications in the laboratory do not significantly alter oxygen transmissibility and are more representative of the slight coatings that were applied in this study. Nevertheless, we measured the Dk/t of 5 to 7 lenses from each of the three lens types that were incubated with protein and dye as described earlier (18 coated lenses in all) and compared the Dk/t with that obtained with an identical number of uncoated lenses. The polarographic method for determining hydrogel Dk/t has been described in detail previously. An electronic thickness gauge (ET-1; Rehder Development Co., Castro Valley, CA) was used to verify that the mean thickness of coated and uncoated lenses were the same. We found no significant difference of Dk/t between coated lenses and uncoated lenses of the same material and thickness.

Procedure

Once the stained lens was inserted, it was allowed to settle on the eye for at least 10 minutes. The subject was seated at the slit lamp and asked to fixate, using the left eye, on an LED placed across the room. The subject was allowed to blink at will. The operator aligned the flash illumination on the center of the cornea and adjusted the PMT voltage to bring the signal on scale with the 12-bit analog-to-digital (A/D) converter of the system (Oxyspot; Harvard Apparatus). Open-eye measurements were then made at 0.5 Hz over 60 seconds and repeated 1 to 2 times to assure that the lens was completely settled on the eye, which was judged by the successive data sets’ being within ±5 torr at a P02 less than 50 torr and ±10 torr at a higher P02. Phosphorescence from the anterior surface of the lens was avoided by including a delay between the flash and data collection. Because the anterior surface is exposed to air (155 torr), its phosphorescence decays very rapidly (half-life, 20 μsec). Therefore, we used a delay of at least 40 μsec.

To estimate closed-eye P02, we asked that subjects close their eyes for 5 minutes while continuing to hold position in the slit lamp after an open-eye measurement. When directed to open their eyes, they immediately took up original fixation. They were instructed to try not to blink for the first 10 seconds and then to blink at will thereafter. Concomitant with eye opening, data collection was started and continued for at least 40 seconds at 1 Hz. Alignment of the flash illumination area with the central cornea was generally preserved, but, occasionally, small adjustments were necessary. In the first 10 seconds after eye opening, most of the individual data point correlation coefficients had to be acceptable to reconstruct the P02 change between the closed-eye and the open-eye condition. If more than two of the data points in this period had correlations that were not acceptable, the procedure was repeated until acceptable measures were obtained. The data collected after eye opening was fit to a first-order exponential model

\[ P_{O2} = SS - (SS - f) \cdot e^{-kt} \]

where P02 is oxygen tension at any time (in torr), SS is the steady state oxygen tension, I is the initial (t = 0) P02, k is the rate constant, and t is time. Regressions were performed using PSiPlot software (Poly Software International, Sandy, UT).

Estimation of Qc

The steady state tear P02 under a contact lens is primarily determined by the corneal oxygen consumption rate (Qc). Thus, given the tear P02 under a contact lens of known Dk/t, it is possible to determine the oxygen flux into the cornea, Jc, which leads to an estimate of Qc. Qc (mL O₂/mL · sec) is calculated from the following equations published by Fatt et al.,

\[ Jc = -\left(\frac{QcLc}{2} + \left( P1 - P2 \right) \frac{Dk0}{Lc} \right) \]

and rearranging:

\[ Qc = \left[ 2(P1 - P2) \frac{Dk0}{Lc} \right] - 2Jc \]

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where \( f_c \) is oxygen flux (in mL O\(_2\)/cm\(^2\)·sec) into the cornea, \( P_i \) is the PO\(_2\) in the tears, \( P_e \) is the PO\(_2\) at the endothelial surface (30 torr), \( L_c \) is corneal thickness, and \( Dk_c \) is the oxygen permeability (in mL O\(_2\)/cm\(^2\)·mL·sec·torr) of the cornea \((2.4 \times 10^{-10})\). At the tear–cornea interface, the flux into the cornea must be equal to the flux of oxygen that is leaving the contact lens, given that the thickness of the tears is relatively small (<10 \(\mu\)m). Therefore, \( f_c = f_{cl} \), and \( f_{cl} \) is calculated by

\[
f_{cl} = \frac{Dk_c}{L_c} (P_{in} - P_{out})
\]

where \( Dk_c/L_c \) is the oxygen transmissibility of the contact lens (Dk/t in the newer notation), \( P_{in} \) is the PO\(_2\) at the anterior surface of the lens and is generally assumed to be 155 torr in the open eye and 55 torr in the closed eye.\(^{16}\) Corneal thickness \( (L_c) \) integrated over a central 3-mm diameter, was measured with a pachometer (Orbscan; Orbtek, Inc., Salt Lake City, UT).

**RESULTS**

In previous studies of tear PO\(_2\) behind rigid contact lenses in rabbits, simple instillation of dye–protein complex solution was made directly into the tears, followed by lens insertion.\(^2\) This worked well in sedated rabbits, because an adequate amount of dye was retained behind the lens for 10 to 15 minutes. Using either rigid lenses or hydrogels, this approach did not work in human subjects, presumably because of more frequent blinking and rapid washout relative to the sedated rabbit. Protein binding to hydrogels is a well-known clinical problem, and the porphyrin dye is completely bound to BSA. We took advantage of this property and bound the dye–protein complex to the lens surface by incubation overnight. Large proteins are not expected to penetrate 38% water hydrogels, and we verified this by sectioning lenses and viewing at \(\times 200\) magnification (data not shown).

The quenching constant, \( q_k \), and the lifetime in the absence of oxygen \((\tau_0)\) of this porphyrin–protein complex are well established.\(^{21-25}\) For the eye–contact lens system we used \( q_k = 504 \) (in torr per second) and \( \tau_0 = 581 \mu\)sec, which are the parameters for this dye at 35°C and pH 7.2.\(^2\) We verified that these parameters were appropriate for our instrumentation. A dye-coated thin hydrogel was placed in saline in a cuvette and kept at 35°C. The saline was bubbled with 100% nitrogen gas or air. The estimated PO\(_2\) in nitrogen was less than 0.1 torr and ranged from 140 to 165 torr in air. To verify that the phosphorescence parameters were appropriate for the lens on the eye, a tight-fitting goggle was placed over the eyes of a subject who was wearing a thin hydrogel dye-coated lens. Humidified nitrogen gas or air was passed through the goggle and the phosphorescence decay measured. Again, for air the estimated PO\(_2\) was 140 to 165 torr. Under nitrogen gas the estimated PO\(_2\) was 0.8 torr. This is a reasonable level, because oxygen diffusion from the anterior chamber and occasional oxygen fluxes from the palpebral conjunctiva during blinks make it difficult to obtain absolute anoxia at the corneal surface.

Figure 1 shows representative open-eye oxygen measurements for the three lenses 10 minutes after lens insertion. Figure 1A shows data from one subject for the high-Dk/t lens taken at 2-second intervals for 60 seconds. The mean ± SD for this data set was 105.6 ± 2.0 torr. Figure 1B shows representative data for the thin hydrogel (mean ± SD; 31.1 ± 1.2 torr). Figure 1C shows data for the thick hydrogel for which the mean ± SD over 60 seconds was 6.7 ± 0.15 torr. These data illustrate that the variability in PO\(_2\) estimates increased with increasing PO\(_2\) and in the four subjects, the average of the SDs of each 60-second data set (30 readings) was 3.85, 2.78, and 0.21 torr, for the Balafilcon (Bausch & Lomb) and thin and thick hydrogel lenses (Metroptics), respectively. Figure 1D summarizes the open-eye data in the four subjects for each lens.

**FIGURE 1.** Tear PO\(_2\) in the open eye. (A–C) Representative data sets of tear PO\(_2\) taken every 2 seconds for 1 minute for the Balafilcon (Bausch & Lomb) and the thin and thick hydrogel (Metroptics) lenses, respectively. (D) Mean open-eye PO\(_2\) in the four subjects for each lens. Error bars, SD.
In preliminary experiments we had subjects who were wearing test lenses close their eyes for 1, 3, 5, and 10 minutes before opening the eyes, to measure the PO₂ to the open-eye steady state value. These experiments indicated that approximately 3 minutes of eye closure was sufficient to establish the closed-eye value—that is, 5 or 10 minutes of eye closure did not produce lower estimates. Therefore, for all closed-eye experiments we used 5 minutes of eye closure to assure closed-eye equilibrium. Figure 2 shows representative data from the same subject shown in Figure 1 for the three lenses, after eye closure. Figures 2A and 2B indicate the initial estimate \( (I) \) of PO₂ for the Balafilcon (Bausch & Lomb) and the thin and thick hydrogel (Metroptics) lenses respectively. (A, B, dashed line) The fit to an exponential model. \( I \) is initial PO₂ at \( t = 0 \), \( SS \) is the steady state PO₂, and \( r^2 \) is the coefficient of determination. For the thick hydrogel lens (C), the first six data points were averaged to obtain the closed-eye PO₂ estimate. (D) Mean closed-eye PO₂ in the four subjects for each lens. Error bars, SD.

**DISCUSSION**

Our goal in this study was to show that human corneal oxygen consumption could be estimated by a direct, noninvasive measure of tear PO₂ beneath contact lenses. Given the oxygen transmissibility of a contact lens, the oxygen flux through the lens can be calculated, because the boundary conditions (an assumed front surface PO₂ and the measured back surface PO₂) are known. In the steady state, oxygen flux out of the lens must be equal to flux into the cornea. From this relation and the corneal thickness, an estimate of \( Q_c \) can be obtained. At high surface PO₂ we estimated \( Q_c \) to be \( 2.2 \times 10^{-4} \) mL O₂/cm² · sec. This is approximately two to three times that reported in dissected rabbit corneas. This difference could be due to species differences or more likely because rabbit corneal oxygen consumption was suppressed by the trauma of explantation to an in vitro measurement apparatus. Weissman and...
Fazio estimated in vivo human corneal oxygen fluxes based on the known lens Dk/t and estimated surface PO2 from corneal swelling experiments. At 25 torr, Weissman estimates human in vivo Qc to be $4.85 \times 10^{-5}$. From our data, the calculated Qc was 5.8 to $6.2 \times 10^{-5} \text{ mL O}_2/\text{cm}^3 \cdot \text{sec}$ at a Po2 of 25 to 30 torr, which is reasonably close to Weissman’s estimate. That Qc decreases with decreasing surface PO2 is not unexpected. Early in vivo studies indicated that at approximately 20 torr, Qc began to decrease. Further, recent mathematical modeling of oxygen distribution from the front to the back surface of the cornea has shown that even at an open-eye surface Po2 of 70 torr, a small portion of the central stroma is anoxic and at surface Po2 between 30 and 40 torr, basal epithelial cells are hypoxic, which would significantly suppress O2 consumption. This study shows that tear PO2 can be measured in human subjects using the oxygen quenching of the phosphorescence probe Pd-meso tetra-(4-carboxyphenyl) porphyrin. The measurement is not completely noninvasive, because it requires hydrogel lens wear. Lens wear itself can have mechanical effects on the surface epithelium that could suppress Qc. This could be tested by determining whether other mild forms of trauma (e.g., surface drying, brief wear of a rigid contact lens, or brief touch with an applanation tonometer) affects Qc. Because of the nature of measuring phosphorescence decay accelerated by oxygen quenching, the measurement is most sensitive at a low Po2. This is exemplified by the greater variability of individual open-eye data sets at a high Po2. The dye–protein complex immobilized to the surface of contact lenses could act as an oxygen sensor for many hours; however, the individual lengths of the experiments in this study were no more than 20 minutes. Binding the dye complex to the lens was needed, because direct instillation into the tears led to rapid loss of signal (<1 minute) due to washout. Protein–dye binding to the lens surface had no effect on lens Dk/t. Also, binding to the lens did not alter the dye’s quenching parameters, presumably because the primary interaction of the porphyrin is with BSA. We assume that the dye complex is indicating Po2 at the lens surface and not from inside the lens. Because BSA has a molecular weight of ~66 kDa it is not expected to penetrate beyond the lens surface. Light microscopy of lens sections indicated that only the surface was stained. Furthermore, the delay between eye opening and a change in measured Po2 when subjects wore the thick hydrogel would have been significantly shorter if dye complex had penetrated the lens. Last, because both front and back lens surfaces are stained, the front surface phosphorescence had to be removed. The front surface of the lens is exposed to air (155 torr) and the decay constant at 155 torr is 20 µsec. Thus significant phosphorescence from the front surface could be avoided by delaying data acquisition after the flash by approximately 40 µsec.

Estimates of closed-eye Po2 have been of interest to contact lens researchers for many years. In the current study, we used closed-eye Po2 estimates to extend the range of surface Po2 to examine its effect on Qc. After eye opening, the fit of the oxygen measurement data to a simple exponential model was very good. Because of the fitting process, the data, especially in the first 10 seconds, had to be of high quality to get an accurate estimate of the initial Po2. In practice, data are often rejected because of poor fixation after eye opening or, more commonly, excessive blinking. However, because of the relatively short time of eye closure, it is convenient to repeat the closed-eye data collection until an acceptable data set is obtained.

Previously, in vivo measurements of corneal oxygen consumption were very cumbersome and somewhat invasive (use of fluid-filled tight fitting goggles) or they were lengthy procedures that relied on the relationship between surface oxygen and corneal swelling. In contrast, the current procedure is relatively simple and quick and has broad applicability. We anticipate that very few human subjects would be unable to tolerate wearing a hydrogel lens for 20 minutes. Within this time, Qc can be determined at two Po2s (open and closed eye). With this technique, the effects of disease, drugs, or surgical interventions on Qc could be determined. For example, it could be used as a measure of the depth of the effects of a disease on the cornea (e.g., diabetes) and of the effects of topical drug use on metabolic activity, recovery of Qc after photoablative surgery, as a determinant in the wound-healing process, or to study the relation between sensory innervation and corneal metabolism (e.g., neurotrophic keratopathy, cataract surgery, or penetrating keratoplasty).

References


