Oxygen Permeability of Collagen Shields

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The oxygen permeability (Dk) of ten 24-hr collagen shields was measured directly by polarographic methodology at approximately 2 hr of hydration. Edge and boundary effects were included in the calculations. Dk was found to be approximately $2.6 \times 10^{-11}$ cm$^2$ ml O$_2$/sec ml mmHg at 35°C. Mean water content of the shields was 65.7% (SD = 1.0%) as measured by a hand refractometer. Therefore, the projected oxygen transmissibility of collagen shields is expected to be compatible with normal corneal metabolism. Invest Ophthalm Vis Sci 31:334-338, 1990

Corneal epithelial metabolism is dependent on atmospheric oxygen reaching the corneal surface through the tears.1 Acute corneal hypoxia induces metabolic changes in the corneal epithelium,2 stromal swelling,3 and physical changes in the endothelial mosaic.4 Chronic hypoxia may lead to microcysts,5 decreased sensitivity,6 thickness,7 and adhesion8,9 in the epithelium; thinning of the stroma;7 and polymegathism10 of the corneal endothelium. However, Mauger and Hill have shown that increased oxygen transmissibility of a contact lens may lead to improved epithelial healing.11

Collagen shields have been introduced as an alternative to bandage soft contact lenses.12,13 We reported recently on the oxygen transmissibility (Dk/L), water content, and thickness of three types of collagen shields,14 but did not directly determine the oxygen permeability (Dk) of the materials. Because the collagen shields are intended to dissolve while in place on the cornea over a period of time (12, 24, and 72 hr), the measurements we made referred to initial hydrated parameters. We found that the Dk/L all three types of collagen shields was similar to that of hydrogel lenses of the same water content (63%) and thickness values; we calculated an initial Dk, predicted from the water content of the collagen, of approximately $2.7 \times 10^{-11}$ cm$^2$ ml O$_2$/sec ml mmHg. The current study extends these results by direct measurement of the Dk of the collagen material.

Materials and Methods

The same polarographic methodology described by Fatt and co-workers15-18 to measure Dk/L for hydrogel contact lenses and materials was used here for measurements of the collagen shields, with the same assumptions and precautions. A polarographic oxygen sensor (no. 8396; Roche Bioelectronics, Boston, MA) consisting of a 4-mm-diameter gold cathode and silver-silver chloride ring anode was mounted on a specially constructed plastic holder. This was connected to a Schema Versatae (Berkeley, CA) 9 20-A amplifier (previously calibrated with three known resistors), which maintained voltage between the anode and cathode at a constant 0.7 v and amplified the resultant current. A linear strip-chart recorder graphed the changes in current, and was used to determine the point in time at which the current became constant.

When an oxygen-impermeable plate of glass was placed on the upper surface of a hydrogel contact lens, collagen shield, or saline-saturated filter paper on this type of polarographic sensor, the cathode consumed all of the oxygen in the lens, shield, or filter. The observed electric current should then have been zero. In practice, there may still have been a small residual current caused by the electrical conduction of saline within the material. This zero-oxygen, or “dark,” current was subtracted from the polarographic current observed during a transmissibility measurement.

The net current was then used in a formula originally proposed by Aiba et al19 to determine Dk/L by the polarographic method. Details have been described elsewhere.14,16 In brief, the calculation of
Dk/L depends upon noting the electron flow (electrical current) necessary to sustain the cathodic reaction

\[ 4e^- + O_2 + 2H_2 = 4OH^- \]  

(1)

The electric current depends on the rate of delivery of oxygen to the cathode. This delivery rate is dependent in turn upon the Dk/L of the sample that is interposed between the cathode and the source of oxygen (e.g., the air above the sample).

Because Dk/L is temperature-dependent, the apparatus was maintained at 35°C, to simulate ocular temperature, by surrounding it with a styrofoam box and by enclosing a 60-W light bulb controlled by an Oven Industries (Mechanicsburg, PA) 5C5-335 thermostat. Paper towels saturated in hospital-grade sterile irrigation water were placed initially on the floor of the box to maintain high humidity so that samples would not change in hydration during the course of the experiment. Five hours were allowed for temperature and humidity equilibration within the box prior to measurement of samples. A fresh bottle of unpreserved 0.9% saline (Hypoclear® Sterile Saline; Bausch & Lomb, Rochester, NY) was kept within the box during this time so that it also would thermally equilibrate.

The observed polarographic current yields an estimate of the Dk/L of the shield. A more exact Dk/L can be obtained only if two measurement artifacts are removed. The first artifact is the “boundary effect.” This artifact arises because it is not possible to eliminate a thin layer of water between the cathode and the sample. Therefore, the measured Dk/L represents the shield in series with a water layer. If a series of shields of the same material but different thicknesses is measured and if the water layer between the shields and the cathode is assumed to remain of the same thickness, then the reduction in observed polarographic current from a thin shield to a thicker shield must represent only the oxygen diffusion resistance of the shield material. Fatt and Chaston demonstrated that the slope of the linear regression between the reciprocal of observed oxygen transmissibility versus sample thickness will be the reciprocal of material permeability and will be independent of the boundary effect.

The second artifact that arises in measuring Dk by the polarographic sensor is a geometrical “edge” effect. The circular polarographic cathode, smaller in diameter than is the sample, creates within the sample a funnel-shaped oxygen diffusion pathway from the air-exposed surface of the sample to an area in contact with the cathode. This effect creates an ambiguity in the application of the relationship described by Aiba et al., because this relationship assumes that oxygen is diffusing through the sample in a right-cylindrical-shaped zone of the same diameter as the cathode. Fatt et al. have examined the mathematics of the funnel-shaped diffusion pathway and have suggested a simple correction factor that reduces the observed polarographic current to the current that would be observed if the sample were a right cylinder of the same diameter as the cathode. They showed that the ratio of the observed current to the current for a right cylinder is given by 1.00 ± 4.72 L (where L is the sample thickness in centimeters) in the case of a sample on a 4-mm-diameter cathode (such as the one used here).

The application of these corrections to the measurement of Dk of collagen shields is complicated by the lack of samples of thicknesses other than about 0.15 mm. Two of us, however, have recently shown that in these measurements stacking samples is equivalent to using a single sample of the same total thickness. Once this premise is accepted, the experiment becomes technically complicated but theoretically direct.

The Fyodorov Collagen Corneal Shield is described by its manufacturer (Bausch & Lomb, Rochester, NY) as a “clear, pliable thin film of porcine-derived collagen in a spherical shell shape.” Ten 24-hr-type shields were supplied dry in small flat plastic containers by the manufacturer for this experiment. We elected to use only one type of shield here, since earlier study showed that all three types were very similar physically. These shields were hydrated in the warmed unpreserved 0.9% saline described above; four 10-ml plastic containers were used, and one shield, two shields, three shields, and four shields were placed into each container, which were filled with 5 ml saline, and then all containers were placed into the styrofoam box for approximately 20 min before measurement.

After equilibration, the collagen shields were removed from the vials and placed (concave surface down) onto a plastic holding tube covered with a nylon mesh. Shields were measured in groups as in the containers, in stacks of one, two, three, and four. Each group was measured twice, with at least 10 min separating each trial for thermal and hydraulic re-equilibration. Care was taken to remove manually any air bubbles trapped between stacked samples prior to measurement. A drop of the preheated 0.9% saline was placed on the electrode surface, and then the tube holding the samples was inverted and inserted into the holder so that the samples were held flat on the electrode. Any residual fluid on the upper surface (over the nylon mesh) was removed with a cotton-tipped applicator prior to measurement.

It took between 2 and 5 min for the polarographic cell current to reach an asymptote with the samples in place. The dark current of our device (measured with a glass slide on top of a saline-saturated cigarette
Table 1. Measured central thickness, water content, and oxygen transmissibility (Dk/L) of stacked collagen shields

<table>
<thead>
<tr>
<th>Number of stacked shields</th>
<th>Central thickness L ± SD (cm)</th>
<th>H2O (%)</th>
<th>Dk/L× 10−9</th>
<th>L/Dk†× 10³</th>
<th>L/Dk‡× 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0152 ± 0.008</td>
<td>66.0</td>
<td>13.64</td>
<td>7.33</td>
<td>7.86</td>
</tr>
<tr>
<td>2</td>
<td>0.0260 ± 0.002</td>
<td>66.2</td>
<td>11.08</td>
<td>9.03</td>
<td>9.67</td>
</tr>
<tr>
<td>3</td>
<td>0.0362 ± 0.000</td>
<td>66.4</td>
<td>9.55</td>
<td>10.48</td>
<td>11.76</td>
</tr>
<tr>
<td>4</td>
<td>0.0412 ± 0.001</td>
<td>64.3</td>
<td>10.91</td>
<td>9.17</td>
<td>10.29</td>
</tr>
</tbody>
</table>

Mean (± SD) 65.7 (±1.0)

* Units: cm ml O2/sec ml mmHg. † Units: sec ml mmHg/cm ml O2.

paper in the place of a sample) was too small to be measured, and should not affect these calculations; therefore, the total measured steady-state current was used to calculate Dk/L for each stack of samples, as has been described previously.14,16

After thermal and hydraulic reequilibration for an additional 5–10 min in each container of warm saline inside the heated styrofoam box, a precalibrated electronic thickness gauge (Rehder Development, Castro Valley, CA) was used to measure the stacked central thicknesses (L),22 and then a hand refractometer (28–62% Brix; Fisher Scientific, Pittsburgh, PA) was used to measure the water content23 of each group of samples. Central thickness was measured four times, with care not to compress the stacked shields; for measurement of water content, the shields were spread out on the surface of the measuring prism to enable reading an average of the surface water contents of as many shields as made up the group.

Two hours of hydration for each group of shields were required to complete the entire experiment; this hydration period is longer than the 1 hr reported for our previous study.14

Results

Table 1 displays the results of measurements and calculations. The thickness of the single shield is in agreement with our previous measurements of 24-hr shields.14 Groups of stacked shields, however, were somewhat thinner than was expected. Effort was made to avoid compression during measurement, and the electronic gauge was specifically designed22 to avoid this type of compression. We must assume, therefore, that our measurements represent true thicknesses of the stacked shields, and that the unexpected thinness reflects the variability in shield thicknesses, which we have also previously reported.14

Some of the variability in thickness seen above might be due to changes in hydration due to increased time in saline. Mean hydration of all four groups of samples in this study was 65.7% (SD = 1.0%), which is slightly higher than our previous measurement.

The reciprocals of measured Dk/L values were corrected for the edge effect by the conversion factor of Fatt et al.,18 as discussed above; these are shown in Table 1 under L/Dk.

Figure 1 shows the relationship between stacked sample thicknesses and L/Dk. The linear regression has a correlation coefficient of r = 0.96 and a form of: L/Dk = (2.1 × 10⁷) + [(3.88 × 10⁹) × L], where L is in centimeters. The slope is 1/Dk, and the reciprocal of the slope is the Dk of the collagen shield material corrected for both boundary and edge effects; Dk is found thereby to be 25.8 × 10⁻¹¹ cm ml O₂/sec ml mmHg.

Weissman and Fatt recently have described an alternative method (called the “one-point” solution) for determining, from a graph such as that of Figure 1, the specific thickness of sample at which the edge and boundary effect corrections should be unnecessary.24 To apply this method, a line is drawn parallel to the linear regression of the edge-effect-corrected data (therefore having the same slope and predicting the same Dk value), but intercepting the origin (as if the boundary effect were negligible). This line intercepts the linear regression of the original uncorrected data at the L and L/Dk values where the edge and boundary effect corrections are not needed. Some algebra is needed to improve precision, but from Figure 1 it can be seen that the above conditions apply to a point defined by an L value of 0.035 cm and an L/Dk value of 13.4 × 10⁷. Taking the reciprocal of this L/Dk value and multiplying by the L value, the Dk of the collagen material is found to be 26.1 × 10⁻¹¹ cm ml O₂/sec ml mmHg.

Discussion

We found previously that the measured Dk/L of the collagen shields was similar to that expected of a
The shields measured here were slightly higher in water content (66%) than those of the previous study, presumably because of the increased time period over which the experiment was conducted, but the measured value of Dk still appeared very similar to that estimated previously. This increase in hydration may have affected the individual thicknesses of the shields discussed above.

The collagen shields examined in the current study are not to be expected to be stable at this Dk value. Hydration and dissolution are expected for these devices as they remain on the eye, and so Dk should increase, and L perhaps decrease, with wear. An initial Dk/L value of about $20 \times 10^{-9}$ was previously reported for the collagen shields, and the results of the current study support this value. Other research with contact lenses suggest that $20 \times 10^{-9}$ is an acceptable Dk/L for daily wear, and perhaps for extended wear as well, since this is only an initial value for these devices, in which Dk/L is anticipated to increase with increasing lens hydration and dissolution over wearing time. The initial parameters of the collagen shield, however, may be interesting to both the clinician using this device and to the laboratory scientist examining the concept.

**Key words:** collagen shields, bandage contact lenses, oxygen permeability, oxygen transmissibility, cornea

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**References**


