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Corneal Equilibrium Flux as a Function of Corneal Surface Oxygen Tension

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Abstract:	Purpose: Oxygen is essential for aerobic mammalian cell physiology. Oxygen tension (pO2) should reach a minimum at some position within the corneal stroma and oxygen flux should be zero, by definition, at this point as well. We sought to explore and discuss in depth this location (xmin) and its physiological implications. Methods: We used our application of the Monod kinetic model to calculate xmin for normal human cornea as anterior surface pO2 changes from 155 to 20 mmHg; Results: We find that xmin deepens, broadens, and advances (in a linear relationship) from 1.25 um above the endothelial-aqueous humor surface towards the epithelium (reaching a position 320 um above the endothelial-aqueous humor surface) as anterior corneal surface pO2 decreases from 155 to 20 mmHg. Only at the highest anterior corneal pO2 does our model predict that oxygen diffuses all the way through the cornea to perhaps reach the anterior chamber. Conclusions: Our model predicts that the epithelial average oxygen flux fraction declines from 0.61 to 0.53 as anterior corneal pO2 declines from 155 to 20 mmHg. Stromal oxygen flux fraction, however, increases over the same range from 0.34 to 0.43. Endothelial oxygen flux fraction is minimal over this range. Corneal oxygen utilization (both consumption and flux) should be supported down to a corneal surface pO2 of 60 to 100 mmHg but may suffer below this range. Thus we would conclude that the critical oxygen tension for hypoxia induced corneal swelling is more likely a range than a fixed value. This also leads us to ponder if the known physiologic oxygen tension of the palpebral conjunctiva (50-60 mmHg) is coincidence.

Equilibrium Dynamic Oxygen Flux Synopsis

Oxygen tension should reach a minimum at some position within the cornea; oxygen flux here should also be zero. With use of a Monod kinetic model, this point is found to deepen, broaden, and advance from close to the endothelial-aqueous surface towards the corneal epithelium as anterior corneal pO_2 decreases. Corneal oxygen consumption and flux should be supported down to a surface pO_2 of 60-100 mmHg but may suffer below this range. Therefore critical oxygen tension for hypoxia-induced corneal swelling is likely a range. This also leads us to ponder if the known palpebral conjunctiva pO_2 is coincidence.

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Corneal Equilibrium Flux as a Function of Corneal Surface Oxygen Tension

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7 Figures and 3 Tables

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ABSTRACT:

Purpose: Oxygen is essential for aerobic mammalian cell physiology. Oxygen tension (pO_2) should reach a minimum at some position within the corneal stroma and oxygen flux should be zero, by definition, at this point as well. We sought to explore and discuss in depth this location (x_{min}) and its physiological implications.

Methods: We used our application of the Monod kinetic model to calculate x_{min} for normal human cornea as anterior surface pO_2 changes from 155 to 20 mmHg;

Results: We find that x_{min} deepens, broadens, and advances (in a linear relationship) from 1.25 μm above the endothelial-aqueous humor surface towards the epithelium (reaching a position 320 μm above the endothelial-aqueous humor surface) as anterior corneal surface pO_2 decreases from 155 to 20 mmHg. Only at the highest anterior corneal pO_2 does our model predict that oxygen diffuses all the way through the cornea to perhaps reach the anterior chamber.

Conclusions: Our model predicts that the epithelial average oxygen flux fraction declines from 0.61 to 0.53 as anterior corneal pO_2 declines from 155 to 20 mmHg. Stromal oxygen flux fraction, however, increases over the same range from 0.34 to 0.43. Endothelial oxygen flux fraction is minimal over this range. Corneal oxygen utilization (both consumption and flux) should be supported down to a corneal surface pO_2 of 60 to 100 mmHg but may suffer below this range. Thus we would conclude that the critical oxygen tension for hypoxia induced corneal swelling is more likely a range than a fixed value. This also leads us to ponder if the known physiologic oxygen tension of the palpebral conjunctiva (50-60 mmHg) is coincidence.

KEYWORDS: Cornea, oxygen tension, oxygen consumption, oxygen flux, Monod kinetics model.

Oxygen is essential for aerobic mammalian cell physiology, wherein 1 mole of glucose reacts with 6 moles of oxygen to form 6 moles of carbon dioxide and water and produces energy in the form of 36 moles of ATP through the Krebs cycle. Cellular oxygen uptake, in general, should remain essentially independent of oxygen tension (pO_2) as long as extracellular pO_2 exceeds a critical value (about 3–6 mmHg[1-4] in general). Below this point, however, it is believed that suppressed O_2 diffusion to mitochondria begins to limit oxidative phosphorylation.[5] Anaerobic metabolism occurs at a cost in efficiency and produces lactate.

Oxygen diffuses through a living cornea down a concentration gradient driven by the difference in its partial pressure between the front and back corneal surfaces according to Fick's law.[6] Allowance, however, must be made for oxygen consumption in each corneal layer (epithelial cells; stroma - primarily keratocytes;[7] and endothelial cells) during this passage. Anaerobic metabolism is common in corneal cells.[8-11]

To understand the potential impact of contact lens (CL) wear to disrupt human corneal aerobic metabolism, oxygen diffusion through the cornea has been studied during the last 50 years and several models have been developed.[12-21] Decreased oxygen consumption suggests that physiology has shifted from aerobic to anaerobic metabolism with the subsequent increase in lactate production leading to increased corneal swelling.[22] By means of both models and experiments, "criticial oxygen tensions" (or COT) have been proposed for various metics. The original COT was defined for CL wear induced corneal swelling (classically an anterior corneal surface pO_2 of 11-19 mmHg[22] but later raised to 70-125 mmHg by other authors for both edema and other metrics[24-29]). The models, however, all agree that oxygen tension should reach a minimum at some position within the stroma (x_{min}). Oxygen flux should be zero, by definition, at this point as well (see below).

The purpose of our study is to first determine x_{min} (where corneal oxygen tension is minimal and flux zero) for the normal cornea under physiological conditions imposed by anterior corneal surface (or cornea-tear interface) pO_2. The second purpose of our work is to explore the relationship of x_{min} as anterior corneal pO_2 changes. Such studies have not been done in parallel and may prove important to our understanding of corneal structure and function, especially when challanged by contact lens (CL) wear.

METHODS

Physiological Parameters

Different authors have considered different parameters to create models of oxygen distribution across the cornea under differing conditions. Table 1 shows the values and units we use in our modeling process including seperate corneal layer thicknesses and oxygen consumptions as well as oxygen tensions at both anterior (cornea-tear layer boundary) and posterior (the endothelial-aqueous humour boundary) corneal surfaces. While some of these parameters have been consistent in the literature over the years, others have varied. For one example, the value of human anterior corneal oxygen flux has been reported in the literature in a wide range, from 1.6 to 10.9 $\mu L \cdot cm^{-2} \cdot h^{-1}$.[30-34]

Insert Table 1 approximately here.

To highlight some specifics, we here use a value of 532 μm for total human corneal thickness, very close to the value of 535 μm of the meta-analysis of Doughty and Zaman[35] instead of previous values (Fatt, for example, often used a corneal thickness of 500 μm as he mostly modeled rabbit cornea[6,12]). We have used a multilayered model cornea with an epithelium 50 μm

thick, a stroma 480 μm thick, and an endothelium 2 μm thick and with specific oxygen consumptions for each layer.[36] We also use a value of 20 mmHg for aqueous humour oxygen partial pressure as found in recent studies[37-40] instead of 55 mmHg.[6,12]

Model for calculation of Balance Flux Region (X_{min})

Corneal oxygen consumption depends on many factors (such as corneal surface pO_2 discussed above, available other nutrients such as glucose, lactic acid, etc.); we here consider corneal oxygen consumption (quantified by a Monod kinetics model[17,20,21]) as a function of anterior corneal surface pO_2. We model a three layer cornea: epithelium, stroma and endothelium. Only monodimensional oxygen flux is considered, and diffusion parallel to the cornea is neglected because the cornea is very thin compared with its width.[6,41-43]

In the steady-state, the equation for corneal oxygen transport can be expressed as

$$\frac{\partial^2 p_c}{\partial x^2} = \left(\frac{Q}{Dk}\right)_c \qquad 0 \le x \le x_c \qquad (1)$$

where x_c, is the thicknesses of the cornea, p_c is the partial pressure of oxygen in the cornea. D is the diffusion coefficient of oxygen in the cornea tissue (cm^2/sec), k is the oxygen solubility coefficient (i.e. Henry's law constant) in (cm^3 O_2 /cm^3 layer/mm Hg), x is the distance perpendicular to the surface in (cm); and Q is the oxygen consumption rate of the cornea (cm^3 (O_2)/cm^3 of tissue layer /sec) as consequence of oxygen metabolic loss. In order to estimate relative oxygen consumption, we considered each corneal layer (epithelium, stroma and endothelium) as having its own maximum oxygen consumption rate, diffusivity, solubility and thickness.

Cornea oxygen consumption is a function of oxygen partial pressure as consequence of aerobic metabolism.[6,20] Aerobic metabolism does not take place at zero oxygen, and therefore at p_c=0 oxygen consumption is also zero. This reaction is also limited at higher oxygen pressures by

the equilibrium concentration of activated complexes formed by reactions between oxygen and the catalytic enzymes. In such cases, the reaction saturates in each layer and oxygen consumption reaches its maximum value $Q_c(p_c)=Q_{max,k}$ (k= stroma, endothelium, epithelium) as indicated in Table 1. Aerobic metabolism in each layer is then quantified by the Monod kinetics model[20] (also known as Michaelis Menten Model[36,44,45]) which relates oxygen consumption with oxygen tension by mean of eq. (2):

$$Q_c(p_c) = \frac{Q_{\max,k} \cdot p_c(x)}{(K_m + p_c(x))} \qquad (2)$$

where K_m is the Monod dissociation constant that determines the shape of the $Q_c(p_c)$ vs. $p_c(x)$ curve and represents oxygen tension when aerobic metabolism reachs its maximum oxygen consumption. Chhabra et al[20] used a Monod kinetics constant of K_m=2.2 mmHg based on oxygen consumption kinetics from transient post-CL tear-film oxygen tensions, assuming that the cornea saturates at 90% oxygen consumption when oxygen partial pressure is p_c=20 mmHg. $Q_{max,k}$ is the maximum corneal oxygen consumption when the reaction reaches the aforementioned steady-state condition, and p_c is the partial pressure of oxygen in the cornea.

Use of the nonlinear Monod model of corneal oxygen consumption described by equation (1) avoids aphysical oxygen partial presures in the cornea (as happens when we assume an constant oxygen consumption rate). The solution of equation (1), taking into account equation (2), has been obtained following the procedure described in the *APPENDIX*.

Whether we work with the model assuming constant oxygen consumption rate (Q=constant), or we use the Monod kinetics model, there will be a minimum in the oxygen tension profile (x_{min}) dependent on the anterior corneal surface pO_2 (where $x=x_c$).[6,39,41]

At x_{min}, $(\frac{\partial p_c}{\partial x}) = 0$ and therefore oxygen flux at this position will also be zero: (J=0). In other words, at the position of minimal oxygen tension in the corneal stroma, oxygen flux also equalizes between "forward" flux from the endothelial-aqueous humor boundary and "backward" flux from the anterior corneal surface. Location of this position will provide more information, however, than just the distribution of oxygen partial pressure throughout the cornea.

Different pO_2 values at the cornea-tears interface (anterior corneal surface), (perhaps simulating CLs of varying oxygen transmisibilities) of 20 to 155 mmHg were considered in this study to evaluate changes in the position of x_{min}.

RESULTS

Figures 1 through 7 show the results of our calculations.

Insert Figure 1 approximately here.

As shown in the left-hand panels of Figure 1, our model suggests that x_{min} both deepens and moves as anterior corneal surface (cornea-tear interface) oxygen tension declines. At the highest anterior corneal surface pO_2 value studied (e.g. equivalent to air at sea level or 155 mmHg), x_{min} is close to the endothelial surface of the cornea, but proceeds forward to about 320 μm from the endothelial surface (perhaps 200 μm from the anterior corneal surface) (Figure 2) and approaches (but does not reach) 0 mmHg at the lowest anterior corneal surface pO_2 evaluated (20 mmHg).

Insert Figure 2 approximately here.

It is interesting to note that only at the highest anterior corneal pO_2 does our model predict that oxygen diffuses all the way through the cornea and perhaps reach the anterior chamber. This has been an area of some discussion over the years.[40,45-48]

We note that, not only does stromal pO_2 decline with anterior corneal surface pO_2, but the stromal tissue exposed to very low (below 3-6 mmHg) pO_2 values broadens.

We calculated oxygen flux (in the epithelium, stroma and endothelium, respectively) from our oxygen tension profiles. Oxygen flux profiles versus depth from the endothelium for different pO_2 values at the cornea-tears layer interface (20 to 155 mmHg) are shown in the right-hand panels of Figure 1. The flux slope at each point in the steady state represents oxygen consumption at this point as shown in equation (3).

$$\frac{\partial J}{\partial x} = -Q(p(x)) \qquad (3)$$

The sum of oxygen consumption rates for each point of epithelium, stroma and endothelium will give the total oxygen consumption value. We thereby determined oxygen consumption for the epithelium, stroma, and endothelium as well as for the total cornea. These results are shown in Table 2.

Oxygen tension and flux profiles for specific anterior corneal surface pO_2 values of 155, 100, and 60 mmHg should be of particular interest. We note that 155 mmHg should be the anterior corneal surface pO_2 of the open eye,[6,12] and 60 mmHg, provided by the palpebral conjunctiva, that of the closed eye;[6,13,39] while 100 mmHg could be a reasonable anterior corneal surface "critical oxygen tension" for swelling during CL wear.[49]

DISCUSSION

If the metabolic model is appropiate, we predict that minimum cornel oxygen (x_{min}) deepens, broadens, and changes location, moving from close to the endothelial surface towards the epithelium as corneal surface pO_2 declines (see Figures 1, 2 and 3). Greater physiological stress and lactate production would be expected in more hypoxic regions. Bonnano and Polse,[50,51] for example, showed a direct relationship between CL-related hypoxia and stromal pH; they felt that the stromal acidosis they detected was likely due to both the production of protons from hypoxic metabolism as well as an accumulation of carbon dioxide.

It is well-accepted that keratocytes are not uniformly distributed but decrease from the anterior to posterior stroma.[52-54] Bergmanson in his text[55] summarizes that stromal keratocytes are most dense just under the Anterior Limiting Lamina, below the epithelium, next most dense just above the Posterior Limiting Lamina (above the endothelium), and least dense in mid-stroma. Our results suggest that there might be a connection (but cause-effect is not clear) between such histological findings and our prediction of lower oxygen availability (and flux) in the posterior-middle stroma under physiological conditions (as noted above, anterior corneal surface pO_2 is expected to be about 155 mmHg with the eye open and about 50-60 mmHg when the eye is closed).

Of interest, several studies document decreased keratocyte density during contact lens wear.[52] Both Erie et al[56] (in keratoconus patients) and Kallinikos et al[57] (in non-keratoconic patients) showed mid-stromal keratocyte density reductions compared to control patients. It might be of clinical concern at this point to note the current use of GP scleral CLs. Despite the higher oxygen transmissibility of current CL rigid materials, GP scleral CLs in vivo create a thick reservoir of tears which should also create anterior corneal surface hypoxia.[58]

Another potential clinical prediction developed from the results of the present study might be represented by stromal ablation during some corneal refractive surgical procedures: LASIK,

PRK, or LASEK. These treatments all thin the corneal stroma, which might imply a backward displacement of x_{min} and an overall increase of oxygen availability, both at x_{min} and throughout the cornea (see Figure 2). This scenario should theoretically predict a maintenance or even an increase in keratocyte density. This result, however, is not supported in the literature; Ali Javadi et al.[59] found a keratocite density ***decrease*** after uncomplicated LASIK surgery. Trauma generated both during the surgical procedure and subsequent healing process, might drive apoptotic effects that result in an overall keratocyte loss irrespective of the new corneal physiological (ie oxygen) conditions following surgery.

Insert Figure 3 approximately here.

Figure 3 shows how oxygen tension within the stroma at x_{min} varies with anterior corneal surface pO_2. Given a specific reasonable COT for keratocyte metabolism with which to compare these values, we might be able to make interesting physiological inferences. If we apply 3-6 mmHg[1-5] (as discussed above) as a local minimal value to preserve cell aerobic metabolism, for example, to Figure 3, we note that an anterior corneal surface oxygen tension of 50-60 mmHg (equivalent to closed eye conditions, ie, sleep), results in just such a minimum stromal oxygen tension.

Insert Figure 4 approximately here.

Of additional interest, Figure 4 suggests that x_{min} varies linearly with cornea-tears interface pO_2 with a correlation coefficient of r^2=0.9958; the position of x_{min} is predicted to change from 1.25

177 μm above the endothelium-aqueous humor surface for an anterior corneal surface pO_2 of 155
178 mmHg (i.e. open eye at sea level), to approximately 320 μm for an anterior corneal surface pO_2 of
179 20 mmHg (perhaps under a low oxygen permeable CL).
180
181 Insert Figure 5 approximately here.
182
183 Others have suggested that anterior corneal oxygen flux is primarily descriptive of corneal
184 epithelial oxygen consumption.[32,34,60] Figure 5 shows that our model predicts an oxygen flux into
185 the corneal epithelium of close to 9 $\mu l(O_2)\ cm^{-2}\ h^{-1}$ under open eye at sea level conditions (anterior
186 corneal surface pO_2 of 155 mmHg), more consistent with the recent results of Takatori et al[34] than
187 the previous results of Jauregui and Fatt.[32] Of interest, Figure 5 also shows that, although the
188 absolute value of flux declines as anterior corneal surface pO_2 decreases, the difference between
189 flux into the epithelium from the tears and out of the epithelium into the stroma has a consistent
190 difference of about 4 $\mu l(O_2)\ cm^{-2}\ h^{-1}$ across the range of anterior corneal oxygen tensions studied;
191 this should represent epithelial layer oxygen consumption alone. Table 3 shows that the epithelial
192 average oxygen flux fraction declines from 0.61 to 0.53 as anterior corneal oxygen tension declines
193 from 155 to 20 mmHg. Stromal oxygen flux fraction increases over the same range, from 0.34 to
194 0.43. Endothelial oxygen flux fraction, however, is minimal over this range with our model and
195 declines from 0.051 to 0.035 between 20 and 155 mmHg.
196 Insert Table 3 approximately here.
197 Insert Figures 6 and 7 approximately here.
198 Figures 6 and 7 plot corneal layer oxygen use predicted by our model in two different
199 manners. Figure 6 compares oxygen consumption (in $x10^{-5}\ cm^3\ (O_2)\ cm^{-3}\ s^{-1}$) to corneal depth, and

suggests that epithelial layer oxygen consumption should be fully supported down to an anterior corneal surface pO_2 of 60 to 100 mmHg, but below this range consumption declines more, implying increased lactate production from anaerobic metabolism. Relatively minimal oxygen consumption occurs below the epithelium. Figure 7 plots predicted oxygen flux (in $\mu l(O_2)\ cm^{-2}\ s^{-1}$) versus anterior corneal surface pO_2. This figure similarly suggests that oxygen flux (for all three layers as well as total cornea) is supported down to cornea-tear interface pO_2 values of 60 to 100 mmHg, Both Figures 6 and 7 therefore support a range rather than a clear-cut COT for stromal edema.[49]

Of course our conclusions are based on the validity of the metabolic (nonlinear Monod kinetics) model, which considers that corneal oxygen consumption is totally a consequence of aerobic metabolism as a function of oxygen partial pressure into the cornea with a K_m=2.2 as proposed by Chaadra et al.[20,21] Other authors, however, proposed a value of K_m=0.5 based on mithochondrial activity of cell respiration under hypoxic conditions[61] which would displace x_{min} towards the aqueous humor. Under such circumstances, one might speculate that there might not be an x_{min} in mid-stroma in the presence of maximum oxygen availability at the corneal surface but perhaps x_{min} would be in contact with the endothelium. It is however very easy to investigate this question from our model. From equation (Ap.3), taking equation (Ap.2) into account, for K_m=0, we derive that the variation of pressure (p_c) against corneal thickness depth gives a $x_{min}>0$ even for open eye conditions at sea level (p_c=155 mmHg).

Using the same criteria of Chhabra et al[20] we also calculated the oxygen deficiency factor (ODF)[21] area for each oxygen tension profile of anterior oxygen tensions: 20, 30, 40, 50 mmHg. The determined ODFs vary between 90.1% and 35.3% when anterior corneal oxygen tension changes from 20 to 50 mmHg, respectively.

Moreover, using previously published particulars of four hydrogel and six silicone hydrogel lenses[62,63] to analyze corneal oxygen consumption distribution (with the Monod kinetic model[64]), our group has shown[65] that the maximum corneal oxygen-consumption rate ($Q_{c,max}$) is not a constant independent of anterior corneal pO_2: $Q_{c,max}$ increases while anterior corneal pO_2 decreases until around 100 mmHg, after which $Q_{c,max}$ decreases for lower pressures.

Such variations could be related to limitations in all the previously cited models (which consider oxygen consumption solely dependent on pO_2). When other dependencies occur, such as acidosis, pH variation, swelling, etc., a transition is possible. That is, when pO_2 decreases, $Q_{c,max}$ initially increases (associated with change in stromal pH) - but $Q_{c,max}$ then decreases with greater pO_2 reductions, possibly due to changes in glucose concentration (related to cellular anaerobic respiration). Therefore other terms could be required to fully describe corneal pO_2 profile behavior. This generalization of Monod model[65] suggests that at least two different processes can modify $Q_{c,max}$ dependence with corneal pO_2. In this sense, models predicting corneal metabolic processes should therefore have at least two or more coupling factors with the intention to describe other phenomena involved in corneal respiration: we suggest at least both pH and glucose concentration. A generalization of the metabolic model used in our study should be performed to acquire a better description of human corneal behavior allowing modification of $Q_{c,max}$.

In summary, if the metabolic model is appropriate, our study suggests that maximum hypoxic corneal stress (x_{min}) moves from close to the endothelium towards the anterior stroma, and both deepens and broadens, as anterior corneal surface pO_2 decreases. Corneal oxygen consumption and flux should be supported down to a corneal surface pO_2 of 60 to 100 mmHg but may suffer below this range. Thus we would conclude that the COT for hypoxia induced corneal swelling is

more likely a range than a fixed value. This also leads us to ponder if the known oxygen tension of the palpebral conjunctiva (of 50-60 mmHg) is coincidence.

We hope our present study promotes discussion of the potential implications of our calculations for corneal physiology and histology, both under normoxic conditions and in the presence of decreased anterior corneal surface oxygen tensions (e.g. during contact lens wear).

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Table 1. Parameters considered to obtain the oxygen tension and flux profiles across the cornea.

Parameter	Symbol	Value	Units
Partial Pressure of Oxygen at the cornea-tears interface (anterior corneal surface)	p_{tc}	From 20 to155	mmHg
Aqueous Humor Oxygen Tension	p_{aq}	20	mmHg
Maximum endothelium oxygen consumption	$Q_{en,max}$	47.78×10^{-5}	$cm^3(O_2) \cdot cm^{-3} \cdot s^{-1}$
Maximum epithelium oxygen consumption,	$Q_{ep,max}$	25.9×10^{-5}	$cm^3(O_2) \cdot cm^{-3} \cdot s^{-1}$
Maximum stroma oxygen consumption	$Q_{st,max}$	2.29×10^{-5}	$cm^3(O_2) \cdot cm^{-3} \cdot s^{-1}$
Stroma oxygen permeability	$(Dk)_{stroma}$	29.5	Fatt Dk unit
Endothelium oxygen permeability	$(Dk)_{end}$	5.3	Fatt Dk unit
Epithelium oxygen permeability	$(Dk)_{epi}$	18.8	Fatt Dk unit
Stroma oxygen diffusion coefficient	D_{stroma}	2.81×10^{-5}	cm^2/s
Endothelium oxygen diffusion coefficient	D_{end}	0.496×10^{-5}	cm^2/s
Epithelium oxygen diffusion coefficient	D_{epi}	1.767×10^{-5}	cm^2/s
Central Corneal Thickness	CCT	532	μm
Epithelium thickness	T_{ep}	50	μm
Stroma thickness	T_{st}	480	μm
Endothelium thickness	T_{en}	2	μm

Fatt Dk units $= 10^{-11}$ (cm^2 /sec)[ml $O_2 \cdot cm^{-3} \cdot mmHg^{-1}$)])

Fatt Dk/t_{av} units = 10^{-9} (cm ml O_2)/(cm^{-3} sec mmHg)

The values of solubilities has been calculated from the values of permeability and diffusion coefficient admiting that P=Dk in each layer.

Table 2: Calculated average oxygen consumption in the corneal layers, and, in the last column, total corneal oxygen consumption, for various anterior corneal surface oxygen tensions (p_{tc}).

p_{tc} (mmHg)	Endothelial $Q_{en}x10^{-4}$ $cm^3(O_2)\cdot cm^{-3}\cdot s^{-1}$	Stromal $Q_{st}x10^{-4}$ $cm^3(O_2)\cdot cm^{-3}\cdot s^{-1}$	Epithelial $Q_{ep}x10^{-4}$ $cm^3(O_2)\cdot cm^{-3}\cdot s^{-1}$	Corneal $Q_{cornea}x10^{-4}$ $cm^3(O_2)\cdot cm^{-3}\cdot s^{-1}$
20	4.28245	0.117	2.022	0.313
30	4.28259	0.141	2.256	0.356
40	4.28295	0.161	2.363	0.384
50	4.28365	0.177	2.421	0.404
60	4.28476	0.188	2.456	0.418
70	4.28622	0.196	2.480	0.427
80	4.28794	0.202	2.496	0.434
90	4.28984	0.206	2.509	0.438
100	4.29183	0.209	2.518	0.442
110	4.29389	0.211	2.526	0.445
120	4.29598	0.213	2.532	0.447
130	4.29809	0.214	2.537	0.449
140	4.30022	0.215	2.541	0.450
150	4.30234	0.216	2.545	0.451
155	4.30339	0.217	2.546	0.452

Table 3. Oxygen average flux fractions for the corneal layers at different anterior corneal surface oxygen tensions (p_c).

p_{tc} (mmHg)	Epithelial J_{epi}/J_{total}	Stromal J_{stroma}/J_{total}	Endothelial J_{end}/J_{total}
20	0.6076	0.338	0.05150
30	0.5956	0.356	0.04520
40	0.5777	0.378	0.04188
50	0.5631	0.395	0.03985
60	0.5275	0.406	0.03856
70	0.5457	0,414	0.03773
80	0.5410	0.419	0.03718
90	0.5378	0.423	0.03679
100	0.5355	0.426	0.03651
110	0.5337	0.426	0.03630
120	0.5324	0.428	0.03614
130	0.5313	0.429	0.03601
140	0.5304	0.431	0.03591
150	0.5297	0.432	0.03582
155	0.5294	0.433	0.03579

Figure Legends

Figure 1. Calculated oxygen tension (left-hand panels) and flux (right-hand panels) profiles versus depth from the endothelium for different pO_2 values at the cornea-tears interface (20 to 155 mmHg). Upper profiles are for anterior corneal surface pO_2 from 60 to 20 mmHg and lower panels show results from 155 to 60 mmHg. These profiles were obtained following the Monod kinetics model considering the layer values for maximum oxygen consumption, diffusion, and permeability given in Table 1.

Figure 2. Calculated corneal oxygen tension (p_{min}) versus depth from endothelial-aqueous humor boundary for the balanced flux point (x_{min}, at which J=0) as anterior corneal surface pO_2 declines from 155 to 20 mmHg (perhaps simulating progressively decreasing CL oxygen transmissibilities).

Figure 3. Calculated oxygen tension at x_{min}, (where there is equilibrium in flux and minimum oxygen availability in the cornea) versus cornea-tears interface pO_2. These results were obtained by a Monod kinetics model with K_m=2.2 mmHg, considering a three-layer cornea (endothelium, stroma and epithelium), each of different values of maximum oxygen consumption rate, oxygen permeability, oxygen diffusivity, and solubility as shown in Table 1. Note that an anterior corneal surface pO_2 of 50-60 mmHg is associated with a minimal stromal pO_2 of about 6 mmHg.

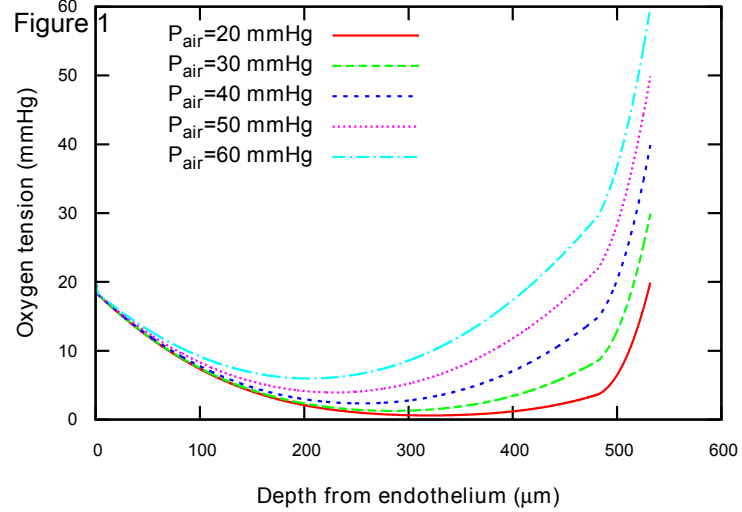
Figure 4. Linear variation of anterior corneal surface pO_2 in mmHg (p_c at $x=x_c$) versus the position of x_{min} in corneal depth from the endothelial-aqueous humor surface. These results were obtained from the Monod kinetics Model with a three-layer cornea, each with different values of maximum oxygen consumption, permeability, diffusivity, and solubility, but the same Monod dissociation equilibrium constant of K_m=2.2 mmHg in each layer.

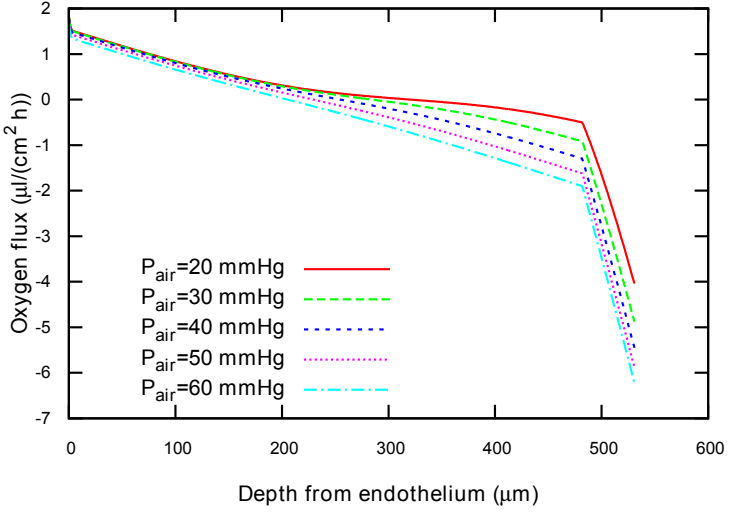
Figure 5. Calculated oxygen flux at both the cornea-tears surface (diamonds) and the epithelium-stroma interface (triangles) versus anterior corneal surface pO_2. Note the difference is consistently about 4 $\mu l\ cm^{-2}\ h^{-1}$.

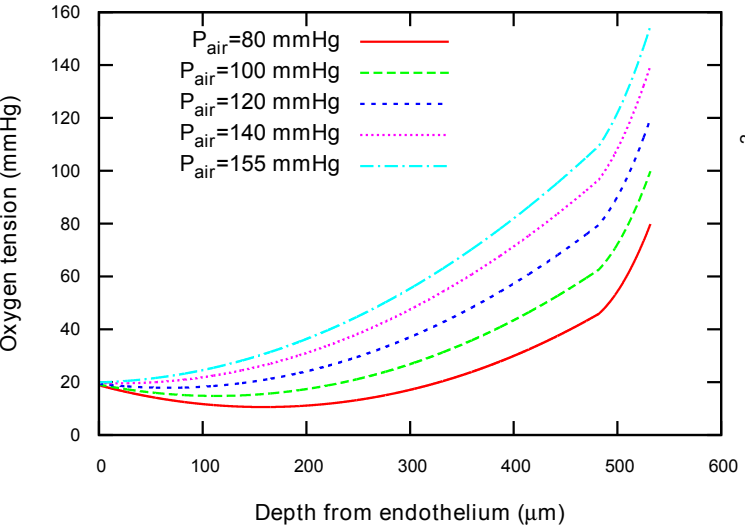
Figure 6. Predicted oxygen consumption (in $x10^{-5} cm^3\ (O_2)/cm^3\ s$) for corneal epithelium, stroma and endothelium as a function of depth from the endothelial-aqueous surface for different anterior corneal surface pO_2 values (20, 60, 100 and 155 mmHg).

Figure 7. Predicted average oxygen flux (in $\mu l\ (O_2)\ cm^{-2}$ tissue s^{-1}) for whole cornea as well as each corneal layer versus anterior corneal surface pO_2 obtained with integration of eq.(3) considering each thicknesses.

Figure 1


Oxygen tension (mmHg)
Depth from endothelium (μm)
Pair=20 mmHg
Pair=30 mmHg
Pair=40 mmHg
Pair=50 mmHg
Pair=60 mmHg


Oxygen flux (μl/(cm2 h))
Depth from endothelium (μm)
Pair=20 mmHg
Pair=30 mmHg
Pair=40 mmHg
Pair=50 mmHg
Pair=60 mmHg


Oxygen tension (mmHg)
Depth from endothelium (μm)
Pair=80 mmHg
Pair=100 mmHg
Pair=120 mmHg
Pair=140 mmHg
Pair=155 mmHg

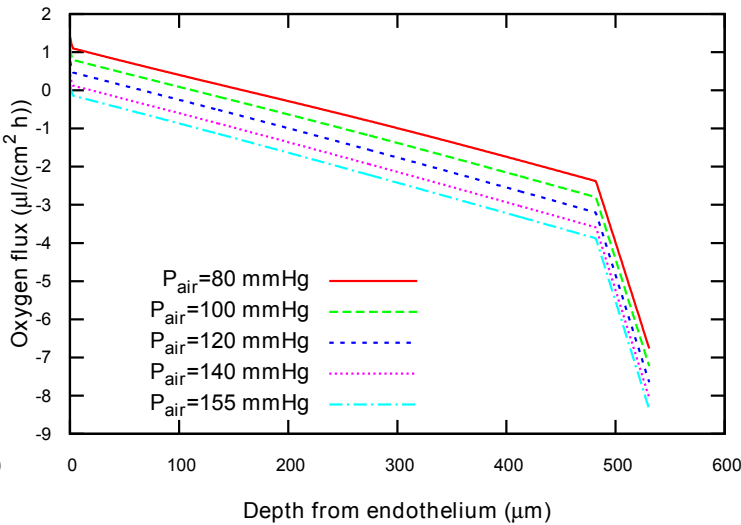

Oxygen flux (μl/(cm2 h))
Depth from endothelium (μm)
Pair=80 mmHg
Pair=100 mmHg
Pair=120 mmHg
Pair=140 mmHg
Pair=155 mmHg

Figure 2

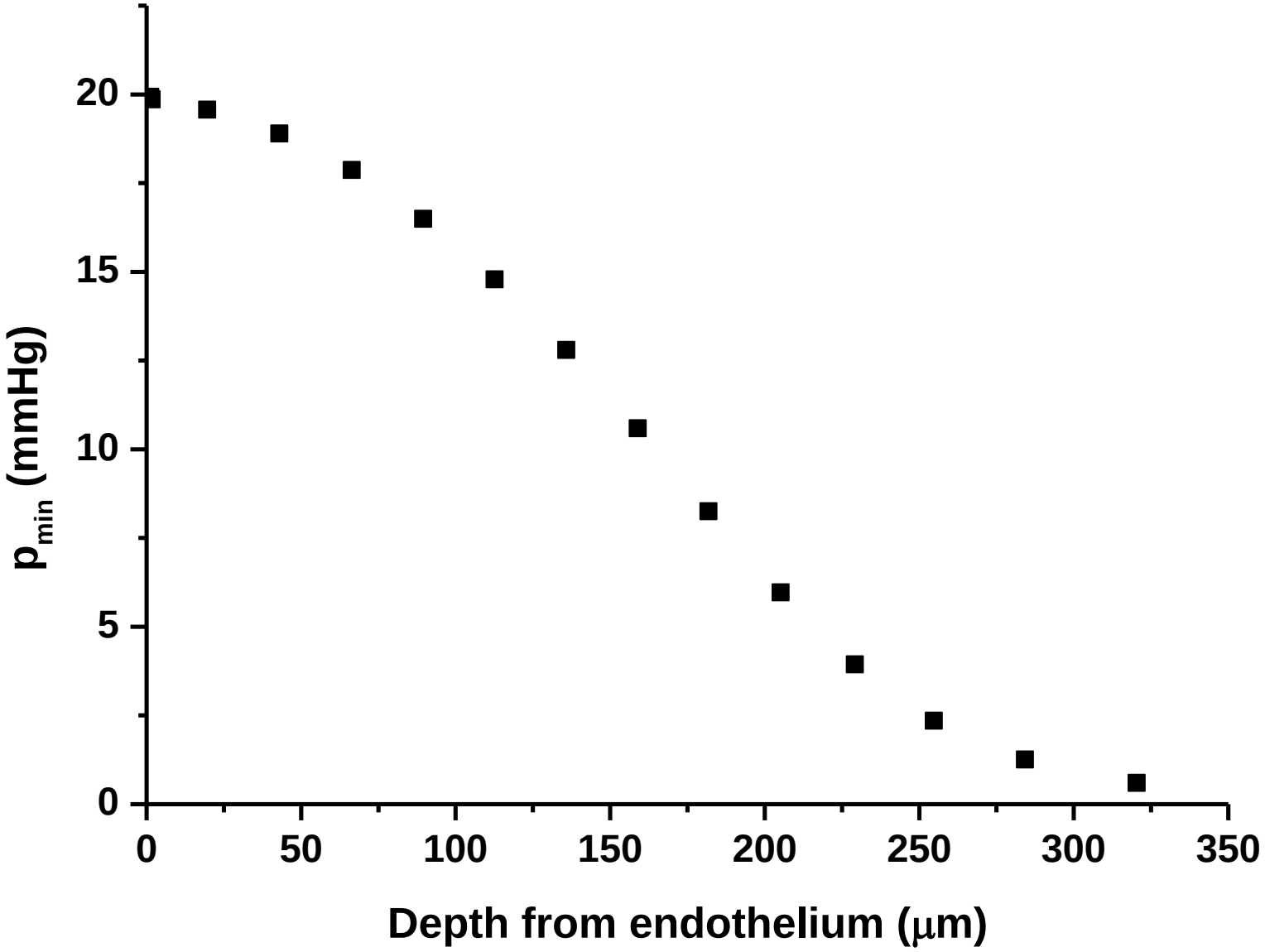

20
15
10
5
0
p_{min} (mmHg)
0
50
100
150
200
250
300
350
Depth from endothelium (μm)

Figure 3

Click here to download Figure Figure 3.tif

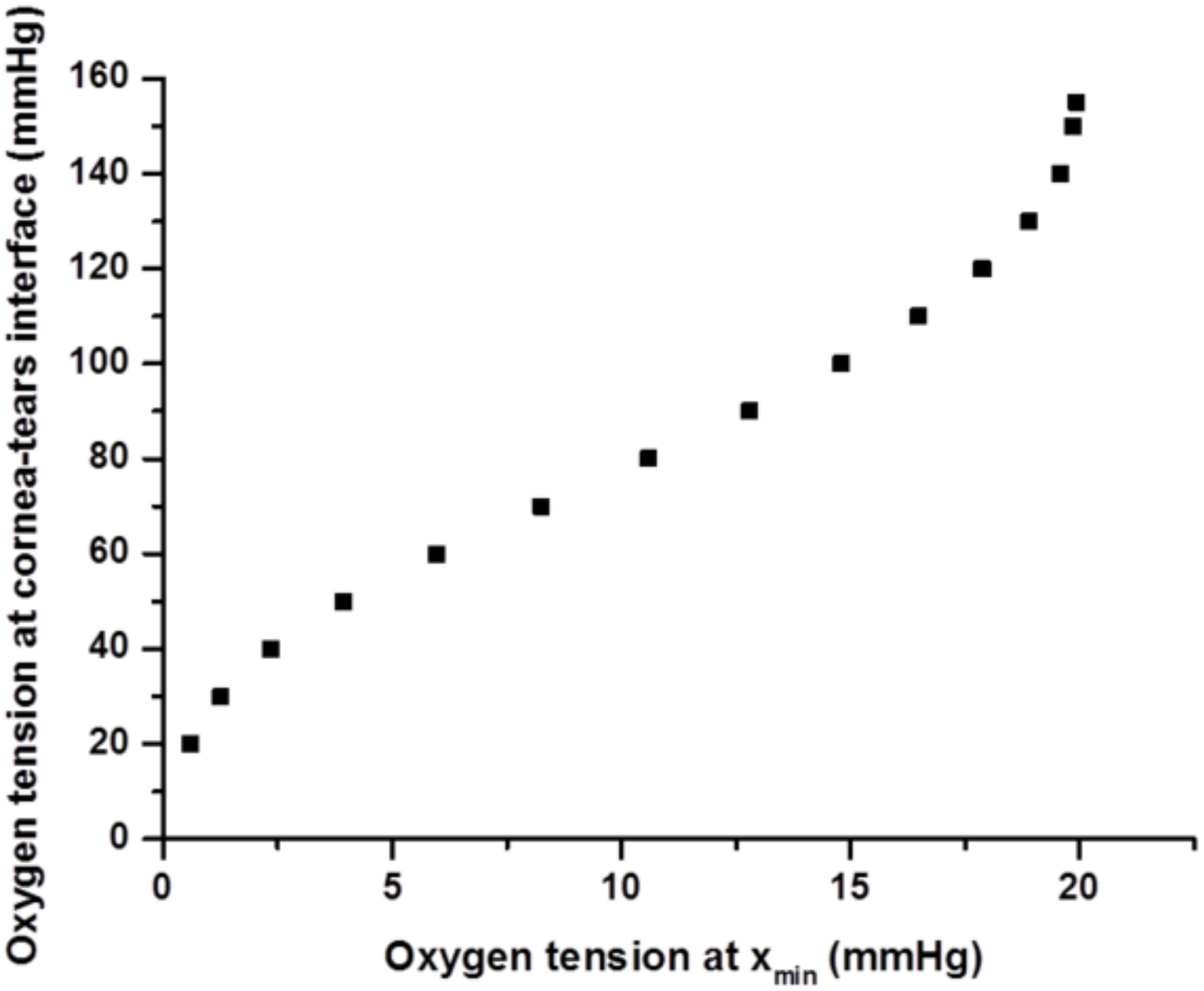


Figure 4

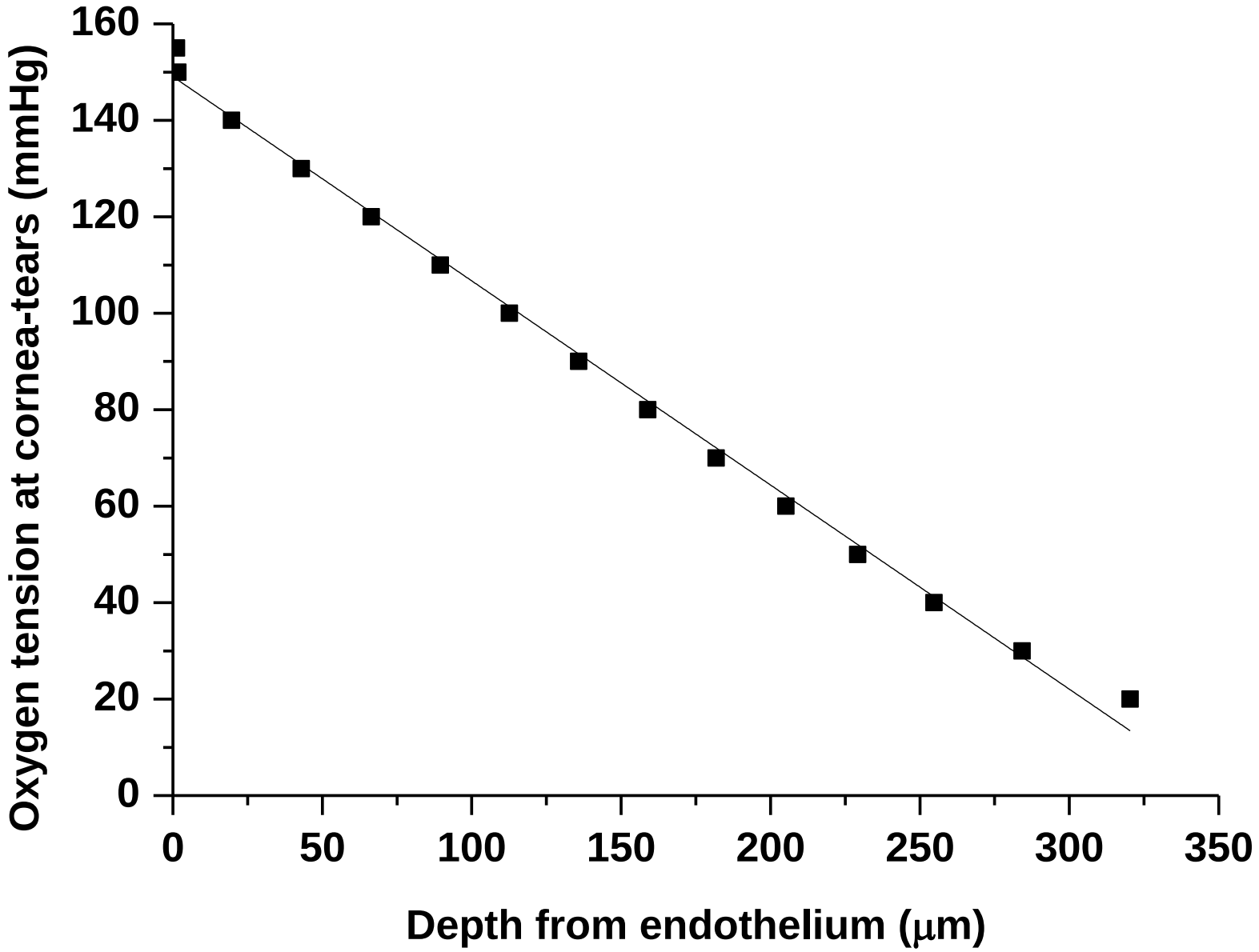

Oxygen tension at cornea-tears (mmHg)
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Depth from endothelium (μm)

Figure 5

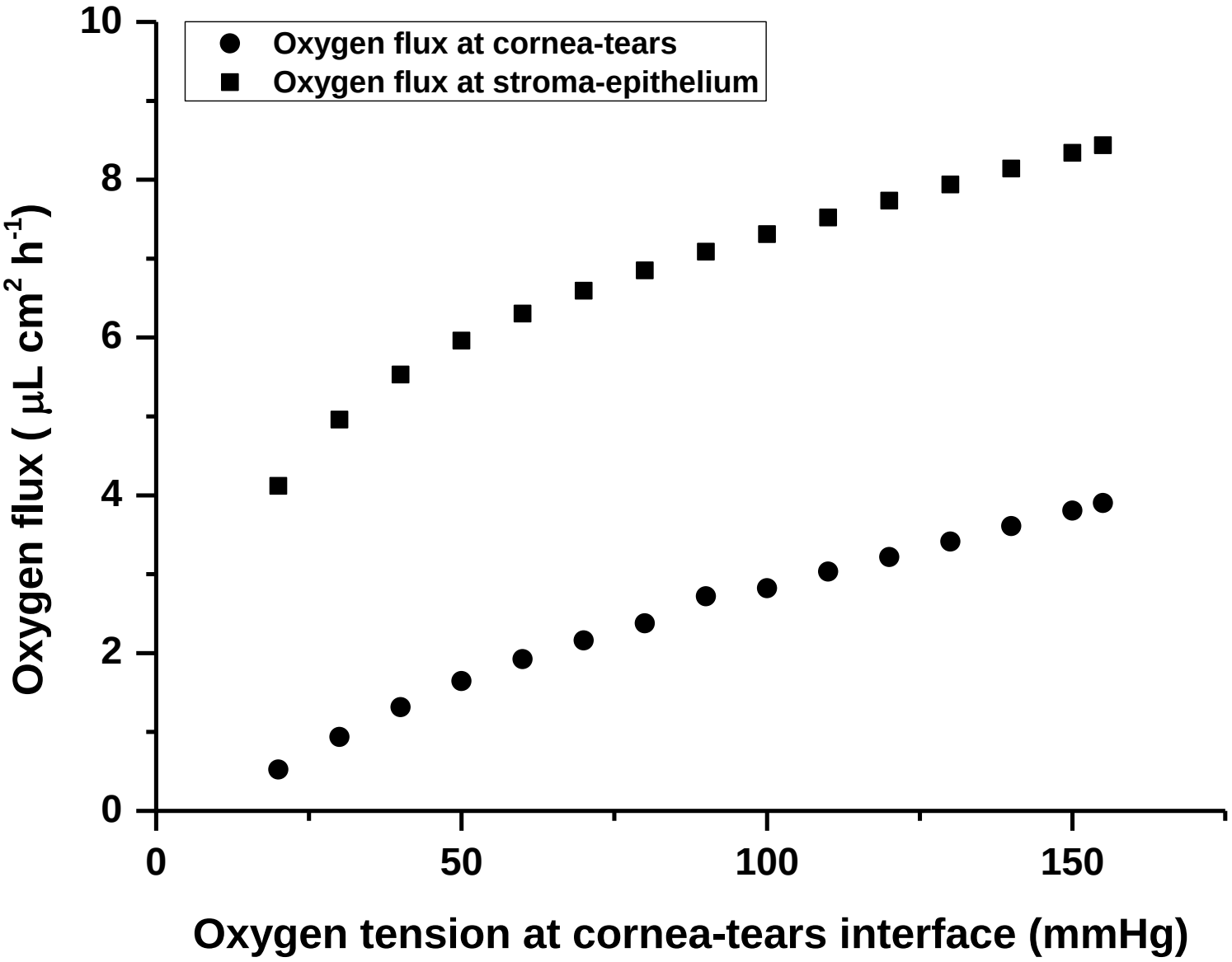

Oxygen flux at cornea-tears
Oxygen flux at stroma-epithelium
Oxygen flux (µL cm^2 h^{-1})
Oxygen tension at cornea-tears interface (mmHg)
0
2
4
6
8
10
0
50
100
150

Figure 6

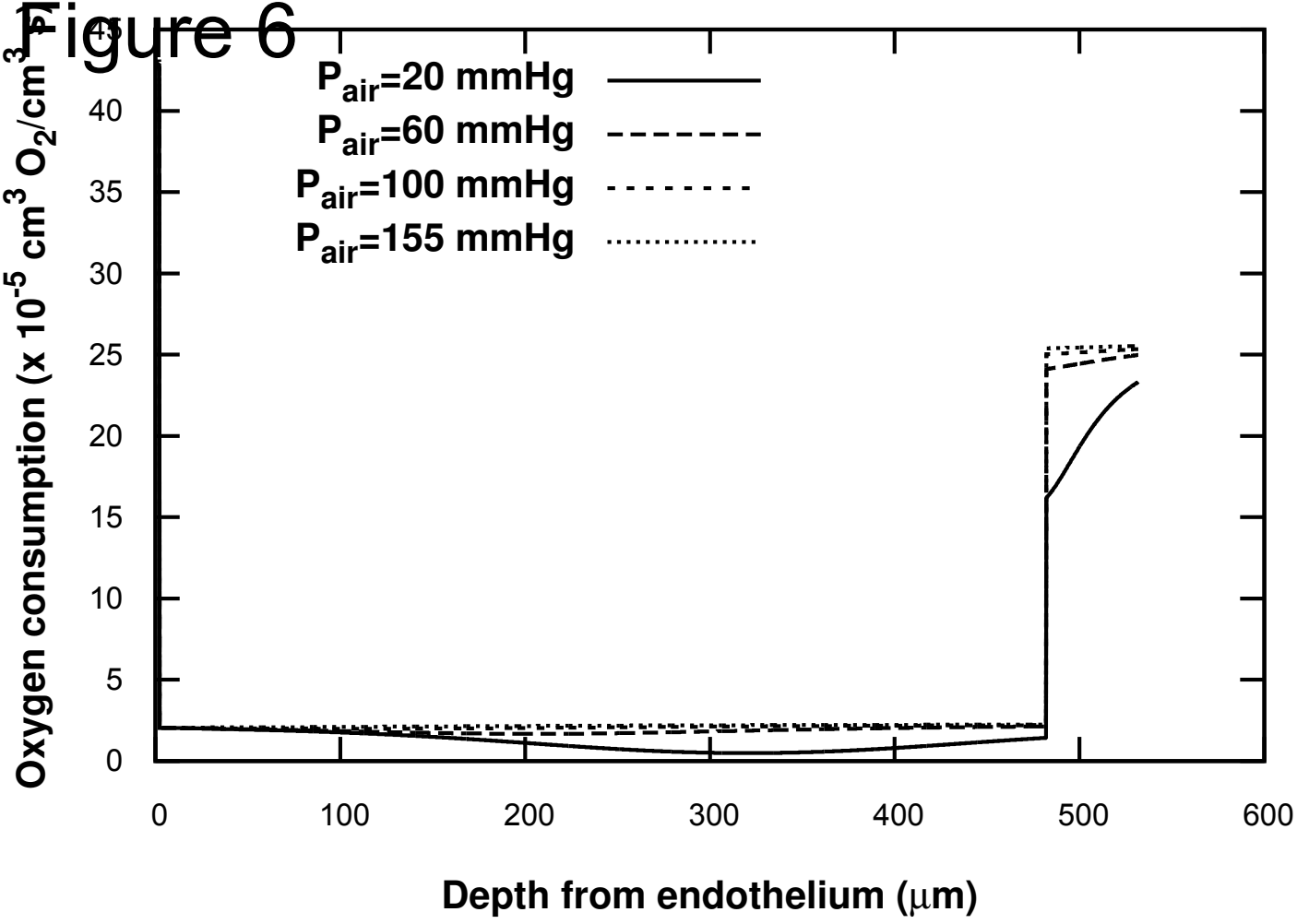
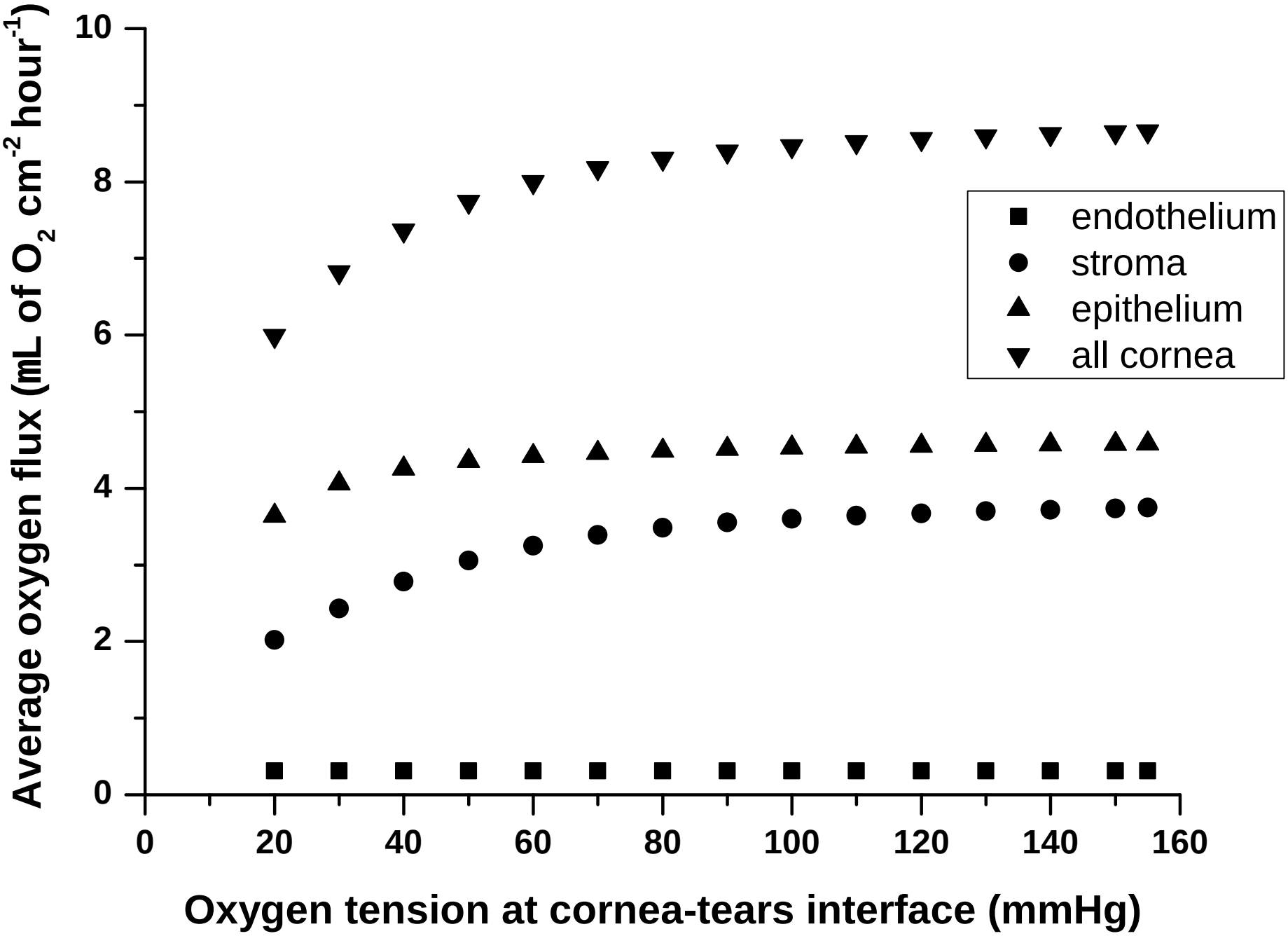

P_{air}=20 mmHg
P_{air}=60 mmHg
P_{air}=100 mmHg
P_{air}=155 mmHg
Oxygen consumption (x 10^{-5} cm^3 O_2/cm^3)
0
5
10
15
20
25
30
35
40
45
0
100
200
300
400
500
600
Depth from endothelium (μm)

Figure 7


Average oxygen flux (mL of O_2 cm^{-2} $hour^{-1}$)
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
Oxygen tension at cornea-tears interface (mmHg)
endothelium
stroma
epithelium
all cornea

APPENDIX

The general equation describing oxygen transport through the cornea system (endothelium-stroma-epithelium) in one dimension, where there is oxygen consumption in each layer, can be described by Fick's second law with reaction[66]

$$k(x)\frac{\partial p(x,t)}{\partial t} = \frac{\partial}{\partial x}\left(k(x)D(x)\frac{\partial p(x,t)}{\partial x}\right) - Q(p(x,t)) \qquad \text{Ap.(1)}$$

where p is oxygen partial pressure, t is time, and x is the coordinate for normal cornea, with x=0 being the interface between the anterior chamber and the cornea. In Eq.(Ap.1), solubility (k) and the diffusion coefficient (D) are considered as a function of position, taking constant values across each of the three regions: endothelium, stroma and epithelium. Equation (Ap1) is reduced in the steady-state condition to the equation:

$$\frac{\partial}{\partial x}\left(k(x)D(x)\frac{\partial P_{est}(x)}{\partial x}\right) - Q(P_{est}(x)) = 0 \qquad \text{(Ap.2)}$$

For times much larger than the characteristic time of the system.

The second term on the right-hand side in Eq.(Ap.1) is oxygen consumption as a function of the partial pressure that follows a Monod kinetics form in the corneal system:[66]

$$Q_c(p) = \frac{Q_{\max,k}\cdot p(x)}{(K_m + p(x))} \qquad \text{Ap(3)}$$

Where k represents each one of the layers (endothelium, stroma and epithelium). By using the above approach, we could obtain the complete pressure profile, provided that the continuity of the pressure is satisfied in the each layer of the cornea. This is automatically satisfied within our numerical scheme.

We chose the standard Dirichlet boundary conditions in the spatial coordinate:

$$P(t,0) = P_{ac} \quad and \quad P(t, x = L_{end} + L_{stroma} + L_{epi}) = P_{ct} \qquad \text{(Ap.4)}$$

where P_{ct} is the partial pressure of oxygen just in the point of the contact of the cornea and tears (the anterior corneal surface). In case of the open eye, this will be atmospheric pressure. In the case of the closed eye, however, this value should be the oxygen tension of the palpebral conjunctiva (i.e

61.5 mmHg). P_{ac} is oxygen tension of the anterior chamber, at the corneal endothelium-aqueous humor surface.

The system of Eq.(Ap. 2-4) are solved using FiPy,[67] a finite volume PDE solver written in Python. Table I shows the different values for the parameters used in the numerical solution of the equations. We used a spatial grid with $2 \cdot 10^3$ points in all computations.

An iterative procedure was used due to the nonlinear nature of the transport equation Ap.2, by "sweeping" the solutions over few iterations (see FiPy manual for details http://www.ctcms.nist.gov/fipy). Convergence was reached after the residual was below a predefined value (10^{-11} in our case). We checked both grid size and time step parameters so that further decrease in size would not result in any improvement. All the computations were performed in a personal computer with an Intel Core i7-3770K under Debian Linux. FiPy version 3.1 was used in all computations.