



Mathematical modeling of ocular epithelial transport: a review

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Abstract

Purpose: Ocular epithelial layers are fundamental for the physiology of the eye as they regulate water transport. The purpose of this review is to discuss the existing mathematical models of water transport across these layers.

Methods: We detail the physical mechanisms that can induce water transport across epithelial layers and describe how they can be mathematically modelled.

Results: We consider 3 ocular epithelial layers. The first is the epithelium of the ciliary processes, which is responsible for aqueous humour production. The second is the corneal endothelium (functionally an epithelium), which plays a key role in maintaining the delicate hydration state of the cornea. The third is the retinal pigment epithelium, which actively removes water from the retina by pumping it into the choroid.

Conclusion: Owing to the difficulty of obtaining direct measurements of water fluxes across epithelial layers, mathematical models can significantly improve our understanding of this field. For instance, they can help develop insight and predictive capability concerning the role of different ion channels, transporters, exchangers, and pumps, as well as carbon dioxide hydrolysis, in ocular water transport processes. Likewise, they can elucidate the importance of the various mechanisms and associated parameters that are involved.

Keywords: epithelial water transport, mathematical modelling, ocular epithelia

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1. Introduction to ocular epithelial layers

1.1 Generalities

Ever since Hooke first stared down a microscope and saw cells, it has been clear that life is formed from compartments. These compartments occur at every scale, from the subcellular, such as mitochondria, to whole organisms. Compartments also exist at the level of organs and the boundaries of these compartments are formed by epithelia, which act both as barriers and as layers performing selective transport. The relative importance and details of these 2 functions vary from site to site. The eye is no exception and has a number of important epithelia.¹ Epithelial layers can be classified into 3 major categories:

1. *Barrier stratified epithelia*, which function as a physical barrier and are multi-layered. The classic example is the skin, but the eye also has such layers: the conjunctival and corneal epithelia.²
2. *Ciliated epithelia*, which perform tangential transport with the help of cilia. An example is the ciliated epithelium of the airway, which generates the mucociliary escalator.³ The eye has no such epithelium.
3. *Secretory epithelia*, which perform vectorial transport. The retinal pigment epithelium (RPE), corneal endothelium, and ciliary epithelium are all examples of this class of epithelia, which transport ions and generate a fluid flow across themselves.⁴ It is these epithelia that form the subject of this review.

1.2 Features of a secretory epithelium

The basic structure of a typical secretory epithelium is a monolayer of cells connected by junctional complexes. The epithelia themselves are avascular and are separated from a supporting vascular stroma by a basement membrane in most parts of the body (Fig. 1). The RPE follows this pattern.⁵ The corneal endothelium (a misnomer, it is really an epithelium) is unusual in that the supporting stroma is avascular; the most extreme deviation from this pattern is the lens epithelium, which completely lacks any supporting stroma.⁶ Epithelial cells are joined by various types of junctional complexes. One example is the adherent and gap junction, which provides mechanical strength and coupling between the cells. The other is the tight junction, which selectively controls the passage of ions. The tight junctions also act to divide the cell membranes into basolateral and apical domains, and this allows the profile of channels, transporters, exchangers, and pumps to differ between these 2 domains, enabling vectorial transport.

Transporting epithelia can be crudely divided into 2 types:

- *Leaky epithelia*: These epithelia support large flows and undertake “bulk” transport but are unable to support large concentration gradients across

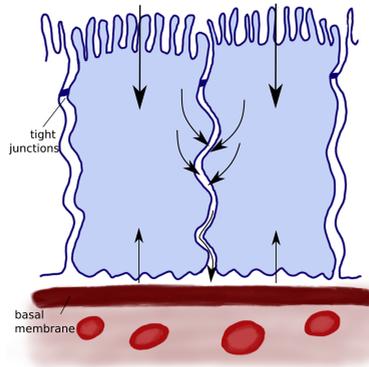


Fig. 1. Sketch of the local osmosis mechanism for water transport across an epithelial layer. The individual epithelial cells are above a basal membrane and capillary supply, and separated by a narrow cleft gap, across which lie the tight junctions that separate the apical from the basolateral regions of the cell membrane. The arrows depict directions of water transport (arrow lengths are not to scale).

them.^{7,8} Consequently, they are leaky since any prospective large gradient of solute will be ameliorated by extensive leaking down the gradient. The small bowel and the renal proximal convoluted tubules are classic examples of leaky epithelia.

- *Tight epithelia:* As their name suggests, these epithelia provide significant resistance to the passage of ions and water, and can support the separation of compartments with a large difference in composition and little paracellular “leakage”.⁹ The epithelia of the large bowel and the distal convoluted tubule are examples of tight epithelia.

In this paper we consider 3 secretory ocular epithelia and review mathematical modeling works that have been proposed to study water transport across these tissues. To start with, in Section 2 and Section 3, we describe the general mechanisms of fluid transport across epithelial layers and the corresponding mathematical models. In Section 4 we address the ciliary epithelium, which lines the ciliary body and separates it from the posterior chamber, located behind the iris. At the ciliary processes, which are ridge-like protrusions from the ciliary body, this epithelium is responsible for the production of aqueous humour, a clear water-like fluid that fills the anterior segment of the eye. The second epithelium we consider is the corneal endothelium (Section 5), located at the inner side of the cornea. This tissue transports fluid from the corneal stroma into the anterior chamber. Finally, we discuss the RPE (Section 6), located at the outermost layer of the retina. The RPE transports fluid from the sub-retinal space into the choroid. We conclude this review by stating open questions in modelling and experiments in Section 7.

2. Mechanisms of water transport across epithelial layers

At the simplest level, the epithelium can be thought of as a permeable membrane and the flow of fluid is simply proportional to the pressure difference across it. If this relationship is linear (doubling the pressure difference results in doubling the flow), it is represented by the following equation:

$$q = L_p \Delta p, \quad (1)$$

where Δp denotes the pressure difference (defined as $p_2 - p_1$, with the subscripts denoting the 2 sides of a membrane). L_p is the hydraulic conductivity of the membrane. In the above expressions, q has dimensions of a velocity (it is a volumetric flux per unit surface from side 2 to 1 of the membrane). Equation (1) can be derived assuming that the flow within the membrane satisfies Darcy flow in a porous medium, and integrating Darcy's law over the membrane thickness.

The next major mechanism that can drive water transport across a membrane is osmotic pressure. Diffusion occurs when particles (or solutes) move from areas of high to low concentration. If these areas are separated by a semipermeable barrier—one that allows water, but not solutes, to pass through—then water will flow in the direction of diluting the compartment with the higher concentration of solute. This is osmosis and leads to the concept “water follows the ions” in the context of epithelial transport. The situation is more complicated in physiology, including ocular physiology. There are 2 separate sets of solutes: ions and proteins (particularly albumen). In order to distinguish these 2 effects, the pressure generated by albumen is called oncotic pressure, Π_p , and we simply refer to osmotic pressure, Π_s , when we consider ions. Fluid flow across a semipermeable membrane is determined by Starling's law, represented in the equation below:¹⁰

$$q = L_p(\Delta p - \sigma_p \Delta \Pi_p - \sigma_s \Delta \Pi_s). \quad (2)$$

where σ_i ($i = p, s$ for proteins and ions, respectively) are referred to as reflection coefficients.¹¹ For an ideal semipermeable membrane, $\sigma_i = 1$, which implies that the membrane is completely impermeable to the solutes; on the other hand, if $\sigma_i = 0$, the membrane is fully permeable to both solutes and solvent and, in this case, no osmotic pressure exists. This equation entails that the flow across the membrane is proportional to the combination of hydrostatic and oncotic/osmotic forces. In Equation (2) the osmotic pressures (both Π_p and Π_s) can be estimated using van't Hoff's law:

$$\Pi = RTc, \quad (3)$$

where c is the molar concentration of osmotic solutes, R the universal gas constant ($\approx 8.31 \text{ JK}^{-1} \text{ mol}^{-1}$), and T the absolute temperature.

Equation (2) has been further extended to account for water transport in the presence of movable and fixed charged solutes and an electric potential difference across

the membrane^{12,13} to obtain:

$$q = L_p \left[\Delta p - \sum_k (\sigma_k RT \Delta c_k - (1 - \sigma_k) z_k F \bar{c}_k \Delta \phi) \right], \quad (4)$$

where the summation is taken over all dissolved chemical species k , with $\Delta \phi$ the jump in the electric potential ϕ across the membrane and Δc_k the jump in the concentration of the k^{th} chemical species. In addition, σ_k and z_k denote the reflection coefficient and valence of species k , F is Faraday's constant, and \bar{c}_k the mean solute concentration through the membrane, which can be computed as the arithmetic average of the concentrations at the 2 sides of the membrane. This equation is an extension of the classical Kedem-Katchalsky¹⁴ model and should be accompanied by an expression for solute fluxes through the membrane.^{12,13}

The above discussion makes it clear that to understand water transport across a biological cell layer, such as epithelium, knowledge about ion movement is required. The problem then is reduced to modeling the movement of ions to generate the associated fluxes. There are 4 mechanisms that account for ion movement and these processes are well understood and can be modeled:

- Molecular diffusion produced by a concentration gradient, which is expressed by Fick's Law.
- Advection due to the flow of fluid.
- Diffusion in response to charged particles moving in an electric field, which can be determined from the electric potential, in turn, given by Equation (9).
- Active transport, and this is performed by the sodium-potassium ATPase pump.

It is known that water is transported across certain epithelial layers also in the absence of significant mechanical pressure, osmotic pressure, and electric potential jumps across the membrane, or when these effects balance each other out. A notable example is the flow across the RPE: in this case, the mechanical pressure jump is negligible and the osmolarity on both sides of the membrane is the same (isotonic transepithelial transport).¹⁵ It is thus clear that, when this happens, additional physical mechanisms must be invoked to explain water motion. To this end, we need to abandon the macroscopic view underlined by Equation (2), which models the epithelium as a semipermeable membrane, and consider the structure of the epithelium at the cellular level.

As pointed out in Section 1, an epithelium is typically a monolayer of cells connected to each other by tight junctions that subdivide the cell membrane into a basal and an apical region. Among adjacent cells, a thin space exists, referred to as "cleft gap". The presence of ion channels and transporters on the cell membrane facing the cell clefts implies that there is a net ion transport from the cell into the cleft gap. These molecules are then transported along the cleft gap by electro-diffusion and, possibly,

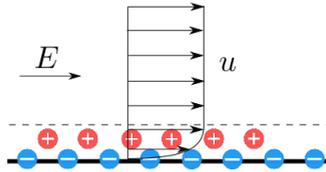


Fig. 2. Sketch for electro-osmosis. Blue circles represent negative charges on the cell membrane that attract positive charges in the fluid (red circles) forming the charged electrical double layer. If an electric field E acts on the fluid, it drags the positively charged fluid, generating a velocity u along the channel.

advection. The result of the above processes is that, in the cleft gap, ion concentrations are typically higher than in the cell. This drives local osmotic water flow from the cell to the cleft, and then a flow along the cleft gaps directed away from the tight junctions. The water flux from the cell to the cleft gap is balanced by an equal net flux into the cell, through both the apical and basal membranes. However, these 2 membranes have different surface areas available for water fluxes owing to the presence of infoldings of the cell membrane, typically on the apical side. Thus, water flux into the cell is larger from the apical side than from the basal side, and this results in net water flux. This phenomenon, which is schematically illustrated in Figure 1, is known as "local osmosis", and was first described in a seminal work by Diamond and Bossert.¹⁶

A final mechanism that cannot be explained using a macroscopic view of the epithelial layer as a membrane is electro-osmosis. An electro-osmotic flow is generated when an electrically charged fluid is acted on by an external electric field. These conditions can be met in the cleft gaps among adjacent cells. The cell membrane is known to be negatively charged and the extracellular fluid contains electrolytes. The bulk of the fluid is electrically neutral; however, the presence of negative charges on cell membranes attracts positive ions in the fluid, with the result that a very thin layer of positively charged fluid forms in the vicinity of the membrane. This charged fluid layer is known as the electrical double layer or Debye layer and typically has a thickness of some nanometers (Fig. 2).¹⁷ Finally, a potential difference exists across epithelial layers, which produces a Lorentz force on the charged fluid in the cleft gap in the longitudinal direction. This can result in a net water flux along the cleft that is balanced by water fluxes across the cell membrane, similar to what is described for local osmosis. This leads to the classical Helmholtz-Smoluchowski theory of electro-osmotic flow.¹⁷ The above mechanisms are illustrated in the sketch depicted in Figure 2.

3. Formulation of the mathematical model

The description of the possible mechanisms involved in water transport across epithelial layers reported in Section 2 makes it clear that fluid transport is inherently associated with the transport of solutes. This implies that water transport must be studied as a multiphysics problem, involving fluid mechanics and the transport of

(potentially charged) molecules that may chemically interact. In this section, we discuss the basic physical principles needed to formulate a mathematical model of water and ion transport; the reader can refer to Table 1 for the corresponding mathematical expressions.

Fluid motion has to satisfy the principle of conservation of mass and Newton's second law (suitably written for a continuum body). In the systems below, hydrolysis of CO_2 produces water; however, the amount is negligible compared to the water concentration in the bulk. Therefore, we assume that there is no fluid production or consumption in the domain. Since in the applications we are concerned with the variations of liquid density with pressure are negligible (the fluid is said to be incompressible), conservation of mass takes the form of a constraint on the velocity field, which is mathematically expressed by Equation (5).

In the case of water transport across epithelial layers, fluid velocities, and length scales of interest are invariably so small that fluid inertia is negligible and Newton's second law reduces to a balance of forces (per unit volume), which is expressed by the Stokes equation (Eq. (6)). In the equation, the first 2 terms derive from surface forces (due to pressure and viscous effects) and the last term (**b**) accounts for external body forces acting on the fluid. The force (per unit mass) acting on an electrically charged fluid by an external electric field can be accounted for through this term.

Solutes are transported by electro-diffusion and advection¹⁰ and the mathematical expression for solute flux is given by Equation (7). Solutes must satisfy a mass conservation principle, which accounts for the transport of a species and its chemical interaction with the others and is expressed by the Nernst-Planck equation (Eq. (8)).

Finally, the electric potential and charge density distribution are linked through the Poisson equation (Eq. (9)), which follows from Gauss' law for electricity. This equation is typically replaced by the condition of electroneutrality in the bulk of the fluid, i.e., at a sufficient distance from charged walls, outside the Debye layer.¹⁸ Electroneutrality is imposed by Equation (10).

The transport of molecules across the cell membrane can be described mathematically using appropriate constitutive equations, which are typically nonlinear functions of the concentrations and, in the case of charged species, of the electrical potential jumps across the membrane. Detailed expressions for such constitutive laws are reported by Dvoriashyna et al.¹⁹

4. Aqueous humor production in the ciliary processes

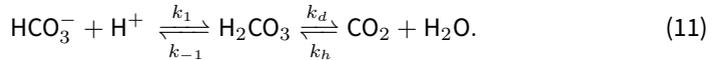
Despite being filled with fluid, the eye maintains its shape due to pressurisation. Excess intraocular pressure (IOP) is a major risk factor for glaucoma, as highlighted in many studies, for example, the Visual Impairment Project.²⁰ The IOP is dictated by the balance between fluid inflow and outflow to the eye, as required for the eye to preserve volume. Specifically, there is a balance between the aqueous humor inflow

Table 1. Equations that govern fluid and ion transport. Table expressions: p : fluid pressure; \mathbf{u} : fluid velocity; μ : fluid dynamic viscosity; \mathbf{b} : body force per unit volume acting on the fluid; f term that accounts for reactions among different chemical species; D and z : diffusion coefficient and valence of the transported species, respectively; F : Faraday's constant; ϕ : electric potential; ρ_e : total volume charge density; ϵ : electric permittivity.

	Mathematical expressions
Fluid mass conservation	$\nabla \cdot \mathbf{u} = 0$ (5)
Momentum equation for fluid motion	$\nabla p - \mu \nabla^2 \mathbf{u} - \mathbf{b} = 0$ (6)
Solute flux	$\mathbf{j} = -D \left(\nabla c + \frac{zF}{RT} c \nabla \phi \right) + \mathbf{u}c$ (7)
Solute conservation	$\nabla \cdot \mathbf{j} - f = 0$ (8)
Equation for the electrical potential	$\nabla^2 \phi = -\frac{\rho_e}{\epsilon}$ (9)
Electroneutrality condition	$\rho_e = 0$ (10)

to the posterior chamber at the ciliary processes of the ciliary body and the outflow of fluid from the eye via both the trabecular meshwork and the uveoscleral routes, as depicted in Figure 3 A, B. Flow through the trabecular meshwork is driven by the pressure drop between the IOP and Schlemm's canal: the higher the IOP, the greater the pressure drop and the greater the outflow.²¹ The uveoscleral outflow is essentially pressure-independent²² and the inflow decreases with IOP, even though this dependence can be weak.²¹ Interventions aiming to reduce glaucoma risk factors thus often reduce the inflow of aqueous humour from the ciliary processes, e.g., via carbonic anhydrase inhibition.²² In turn, this has motivated modeling studies analyzing ion channels, transporters, exchangers, and the Na^+ - K^+ pump, that regulate the inflow from the ciliary processes via the secretory ciliary epithelium, together with the associated osmotic, oncotic, and hydraulic pressures.^{21,23,24}

In particular, the ciliary epithelium lining the ciliary processes of the ciliary body (Fig. 3A-C) consists of a double layer of epithelial cells, with the pigmented epithelium adjacent to the stroma and the nonpigmented epithelium facing the posterior chamber, as illustrated in Figure 3D. A schematic for the ion channels and transporters is depicted in Figure 3E with the following ions and molecules considered in addition to water: Na^+ , K^+ , Cl^- , HCO_3^- , H^+ , CO_2 and H_2CO_3 . Furthermore, the solutes HCO_3^- , H^+ , CO_2 , and H_2CO_3 are subject to the carbon dioxide hydrolysis and hydration reactions:



The fundamental modeling principles outlined previously apply, in particular electroneutrality and that the solutes and water are neither created nor destroyed once tracked through the changes associated with the hydrolysis and hydration reactions (Eq. (11)). Under this assumption, the electrical charge ρ_e , which is defined as the sum of all mobile charges ($z_i c_i$ for each charged solute i) and immobile charges (denoted by X), is zero, $\sum_i z_i c_i + X = 0$.

Recent models based on these principles have been constructed by Sacco et al.²³ and Dvoriashyna et al.,²⁴ together with a lumping of the 2 epithelial layers into a sin-

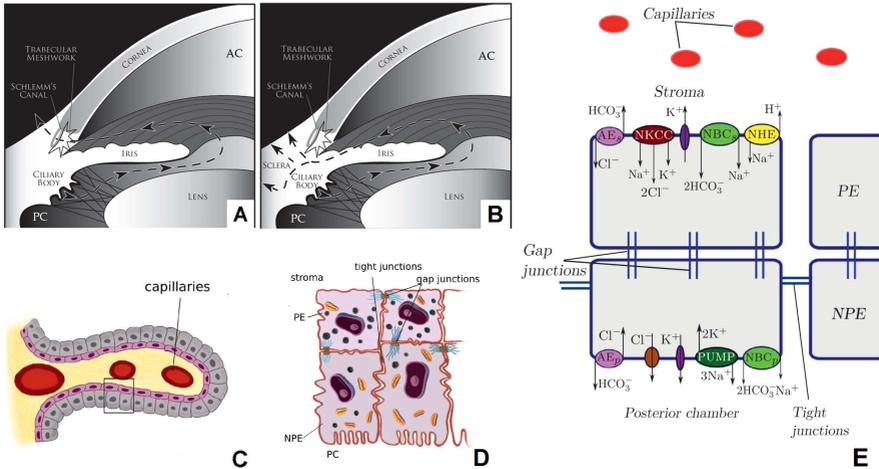


Fig. 3. (A, B) Schematics of the aqueous humour flow, from the ciliary processes (ridge-like structures at the surface of the ciliary body) into the posterior chamber (PC) and then into the anterior chamber (AC), to the outflow through the trabecular meshwork and Schlemm's canal (A) as well as the uveoscleral flow pathway (B). (C) The ciliary process, with its bilayered ciliary epithelium and capillaries on its stromal side. (D) Highlight of the bilayer structure of the ciliary epithelium, with the pigmented epithelium (PE) on the stromal side and the non-pigmented epithelium (NPE) on the posterior chamber (PC) side. (E) The pumps and transporters in the model by Dvoriashnya et. al.²⁴ and their locations. Figures A, and B reproduced from Goel et. al.²⁵, with additional labelling. Figures C-E reproduced from Dvoriashnya et. al.²⁴ under the Creative Commons Attribution Non-Commercial License (3.0, A,B; 4.0, C-E).

gle cellular compartment, as may be motivated by noting that the 2 epithelial layers are connected by gap junctions, thus forming a functional syncytium. In addition, both Sacco et al.²³ and Dvoriashnya et al.²⁴ neglected the additional transport mechanisms discussed in the previous sections, for instance, local osmosis. However, given there is a concentration drop across the ciliary epithelium, one might anticipate that the osmotic flux is the primary mechanism for aqueous humour inflow. In particular, this is indirectly supported by an a posteriori consideration of the predictions of Dvoriashnya et al.²⁴ which, for baseline models and physiological parameters, aligns with measured inflow rates without the need to consider additional transport mechanisms. This is in distinct contrast to the RPE, where at least one mechanism of local osmosis and electro-osmosis is indicated as active, as discussed in Section 2.

In the model constructed by Sacco et al.²³ a subset of the ion channels was considered, neglecting the $\text{Na}^+ - \text{HCO}_3^-$ cotransporter, to predict intracellular concentrations given prescribed ion concentrations in both the stroma and posterior chamber. In particular, this study demonstrated a proof of principle that relatively simple models can relate phenomena at the cellular scale –which are difficult to assess– to behaviour at the level of the eye. However, fixing the concentration jump across the ciliary ep-

ithelium pins the osmotic contributions to the flux of aqueous humour into the posterior chamber, as in Equation (2). However, modulating an ion channel, transporter or the rate of the dissociation reactions of Equation (11) will change the osmotic jump and thus its contribution to the aqueous humour inflow.

Dvoriashyna et al.²⁴ thus constructed a steady-state model without fixing the posterior chamber concentrations and including the $\text{Na}^+\text{-HCO}_3^-$ cotransporter, resulting in 18 algebraic equations for the same number of unknowns. After confirming model consistency with the current understanding, a subsequent sensitivity analysis was conducted. This considered a large number of cases across parameter space to determine predictions for the ion channels and transporters that aqueous humour inflow is most sensitive to. This revealed that inflow was, for instance, significantly more sensitive to perturbations in the dynamics of the $\text{Na}^+\text{-H}^+$ exchanger and the K^+ and Cl^- channels compared to any other ion channel or transporter. Furthermore, carbonic anhydrase dramatically catalyses the second of the reactions of Equation (11), increasing the rate by a factor of 10^6 , so that its inhibition was modeled by reducing k_d , k_h for these reactions by a factor of a million. In turn, this was predicted to induce a reduction of the inflow by approximately 40% (in agreement with experimental observations²⁶) as well as, for example, reducing the ion flux induced by the $\text{Na}^+\text{-H}^+$ exchanger by a factor of 7 or more. Thus, such modeling not only allows one to explicitly assess how our understanding of epithelial membrane transport translates to behavior at the ocular level, but also suggests targets for glaucoma risk factor intervention and provides a detailed theoretical mechanism for how carbonic anhydrase inhibition affects IOP reduction.

Finally, we also remark that Kiel et al.²¹ previously reviewed the biophysics of aqueous humour production prior to constructing a complex model, summarized by a network with extensively more than 100 edges. These authors also confirmed that such a model was consistent with current understanding and highlighted that ciliary blood flow influences aqueous humour, albeit saturating above a critical level of perfusion that is contingent on the level of secretory stimulation or inhibition.

More generally, these 3 models illustrate that the fundamentals of aqueous humour production can be captured by modeling frameworks. Furthermore, this review summarizes how this inflow can be theoretically analyzed to assess how it is predicted to be influenced by modulating membrane transport, thus generating insight into the mechanism of action of current glaucoma treatments and evidence-based hypotheses for the impact and mechanism of action of further prospective interventions.

5. Transport across the corneal endothelium

The cornea is composed of several superposed layers, which are from outermost to innermost:

1. the corneal epithelium;
2. the acellular Bowman's layer;

3. the stroma (which is by far the thickest);
4. Descemet's membrane; and
5. the corneal endothelium, which is actually an epithelium but it is named differently to distinguish it from the outermost corneal layer.

The corneal endothelium is a secretory epithelium that consists of a single cell layer on a basement membrane (Descemet's layer). This epithelium is relatively leaky and thus significant paracellular ion and water transport is possible. Ion transport across the cell membranes is regulated by ion channels and transporters that are differently distributed in the apical (facing the anterior chamber) and basolateral (on the stromal side) membranes.^{4,27} Specifically:

- the basolateral membrane of the endothelium contains the $\text{Na}^+\text{-K}^+$ ATPase pump, the $\text{Na}^+\text{-H}^+$ exchanger, the $\text{Na}^+/\text{K}^+ / 2\text{Cl}^-$ cotransporter and K^+ channels;
- the apical membrane contains Na^+ channels and the $\text{Na}^+\text{-HCO}_3^-$ cotransporter.

The cornea is the first ocular structure that light rays have to cross to reach the retina and thus needs to be transparent. Such transparency depends on the stroma being in a proper state of hydration ($\approx 78\%$ of water content).^{28,29} This happens through a "pump-leak" mechanism, as extensively detailed in Klyce's review.³⁰ In summary, the pump is represented by the corneal endothelium,³¹ which actively removes water from the corneal tissue and transports it into the anterior chamber. The "leak" consists of water transport in the opposite direction and is produced by the stromal swelling pressure,³² i.e., its tendency to adsorb water as a consequence of repulsion forces exchanged by negatively charged stromal glycosaminoglycan. These 2 mechanisms act in opposite directions, without a net water flux being generated. Water exchange between the anterior chamber and the stroma is of utmost importance for the physiology of the corneal tissue. This is because the stroma mostly receives nutrients from the aqueous humour. Moreover, a significant amount of lactate is produced by the stroma, which has to be removed and transported into the aqueous humour.

Leung et al.³³ and Cheng and Pinsky³⁴ have proposed mathematical models of corneal metabolism. The authors modelled water and solute transport adopting a macroscopic view and employing Kedem-Katchalsky-type membrane conditions,^{12,14,33} as seen in Equation (4) for the water flux. In both works, the membrane condition for solute flux includes an additional term that accounts for the active pumping of the bicarbonate ion, directed against its chemical potential. These models consider the pump-leak functioning of the corneal tissue and are capable of reproducing stromal tissue swelling in response to hypoxia.

In normal conditions, water transport from the stroma to the aqueous humour is almost isotonic and therefore, based on the discussion reported in Section 2, either local osmosis or electro-osmosis are likely to play a role. No evidence yet exists on the role of local osmosis in water transport by the corneal endothelium. On the other hand, some experimental work points to the importance of electro-osmosis. Sanchez et al.³⁵ showed with *ex vivo* experiments on rabbit corneas that water flow could be generated across the endothelium by applying a potential difference across the tissue and that the direction of such a flow was reversed upon reversal of the potential difference. Moreover, they showed that water flux was proportional to the intensity of the electric current. As discussed in Section 2, for electro-osmosis to work *in vivo*, a transepithelial potential difference must exist. A potential difference of ≈ 0.5 mV is indeed measured across the corneal endothelium (negative potential on the apical side), which confirms the possibility that electro-osmosis might be a key player in water transport.

To our knowledge, the only attempt to develop a mathematical model of electro-osmotic transport across the corneal endothelium is due to Rubashkin et al.³⁶ The authors developed a model of flow in a channel with charged walls, based on the classical Helmholtz-Smoluchowski theory, the channel being represented by the cleft gaps among cells. Using realistic values of the model parameters, they estimated a flux across the epithelium that is an order of magnitude smaller than that measured in experiments in the same conditions. As an alternative transport mechanism, Rubashkin et al.³⁶ also considered the possibility that the electro-osmotic flow is produced within the leaky tight junctions connecting adjacent cells. The Helmholtz-Smoluchowski theory was suitably modified, treating the flow within the tight junctions as a Darcy flow in a porous medium with charged walls. In this case, owing to the small size of the tight junctions, the electric field acting on the fluid is relatively large, as the electric potential drop occurs across the very short thickness of the tight junction; the water flux predicted by the model in this case agrees well with experimental observations.

6. Transport across the retinal pigment epithelium

The RPE is the outermost layer of the retina, which separates photoreceptors and the subretinal space from Bruch's membrane and the choroid.³⁷ It is a monolayer of cells arranged as hexagonal tiles. The cells are connected by tight junctions on the side of the subretinal space, which separate the cellular membrane and the extracellular space into the apical (the subretinal space) and the basolateral regions.⁵ Between the cells there is the so-called cleft gap, a thin space approximately 20 nm thick. At the basal region there is Bruch's membrane and the choroid.

The RPE is a secretory epithelium and one of its main functions is to transport fluid and solutes from the subretinal space to the choroid.³⁷ The rate of fluid transport across the RPE ranges from 4 to 11 $\mu\text{l}/\text{cm}^2/\text{h}$.^{38,39} Failure of this transport may lead to

fluid accumulation in the subretinal space, which is associated with several pathologies, such as age-related macular degeneration, diabetic macular edema, and retinal detachment.⁴⁰

The mechanisms and pathway of water transport across the RPE are not entirely understood.⁴¹ There is no appreciable pressure or osmolarity difference between the apical and the basal sides of the RPE,⁴ suggesting that the possible mechanisms of transport are local osmosis and/or electro-osmosis.

Recently, we developed mathematical models to quantify these mechanisms and investigate their relative importance.^{42,43} The ions with largest concentrations in the RPE are Na^+ , K^+ , Cl^- , and HCO_3^- . The transepithelial transport of these ions has been studied *in vitro* in various species and the underlying mechanisms have been quantitatively identified,^{40,44,45} as shown in Figure 4.

In the first simplified model,⁴² we consider the presence of 3 ions (Na^+ , K^+ and Cl^-), and account for the possibility of both local osmosis and electro-osmosis in the cleft gap. The model considers the cellular compartment and the cleft between cells and is based on conservation of electrodiffusive flux for each ion, electroneutrality, and balance of water flux (Table 1). We found that active ion transport leads to solute accumulation in the cleft gap that drives a local osmotic flux towards the choroid, according to the mechanism shown in Figure 1. The magnitude of this flow is comparable to experimentally measured values. Electro-osmosis, on the other hand, was found to be several orders of magnitude smaller than local osmosis. The RPE is classified as a tight epithelium and there is a potential jump of about 15 mV across the cell layer. However, this jump occurs mostly across the tight junction, and we found the electrical potential drop across the cleft gap to be only 0.1 mV, which is too small to drive appreciable electro-osmotic flow.

The model above was later extended⁴³ to account for the presence of other solutes in order to assess the importance of various ion channels and obtain a more comprehensive picture of water and solute transport. In particular, we considered: Na^+ , K^+ , Cl^- , HCO_3^- , H^+ , CO_2 , and H_2CO_3 and the latter 4 of these solutes were once more subject to the hydrolysis and hydration reactions of Equation (11), as also considered for aqueous humour production above.

The model incorporated the transport of solutes across the cell membrane through the ion channels and transporters shown in Figure 4, and predicted values of ion concentration and electrical potential similar to those observed experimentally. It also confirmed that local osmosis was the dominant mechanism for fluid transport. In order to study the dependency of water flux on the model parameters, particularly amplitudes of the ion channels, a global sensitivity analysis was performed. The key ion transporters were found to be Na^+ - H^+ exchanger and Na^+ - K^+ ATPase. Inhibition of these transporters in the model led to the prediction of a significant decrease in water flux towards the choroid. A clinical implication of these findings is that fluid accumulation may result as a side effect of Na^+ - H^+ exchanger inhibitors, such as amiloride, or in response to reduced activity of Na^+ - K^+ ATPase due to low metabolic activity.

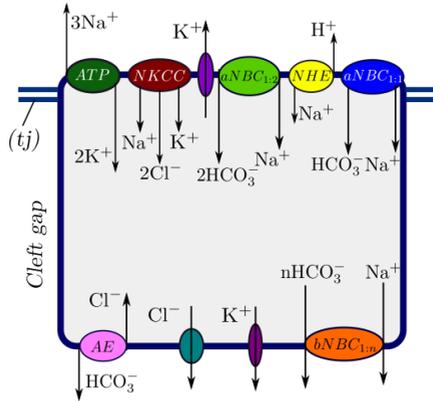


Fig. 4. Sketch of a retinal pigment epithelium cell. The subretinal space is at the top of the cell, while Bruch's membrane is at the bottom. The membrane ion channels are shown with coloured ovals. (Top) Apical membrane and (tj): tight junction; *ATP*: Na⁺-K⁺ ATPase; *NKCC*: Na⁺-K⁺-2Cl⁻ cotransporter; *aNBC_{1,2}*: Na⁺-2HCO₃⁻ cotransporter; *aNBC_{1,1}*: Na⁺-HCO₃⁻ cotransporter; *NHE*: Na⁺-H⁺ exchanger; K⁺ channel. (Bottom) Basolateral membrane. *AE*: Cl⁻-HCO₃⁻ exchanger; *NBC_{1:n}*: Na⁺-HCO₃⁻ co-transporter with stoichiometry 1:n.

7. Conclusions and future modeling perspectives

Epithelial layers play a fundamental role in the physiology of the eye as they regulate water transport phenomena. In this paper we have reviewed the characteristics of the main ocular epithelial layers (the ciliary epithelium, the corneal endothelium, and the RPE), focusing our attention on mathematical models of water transport.

The small spatial scales involved imply that performing direct measurements in vivo and even in vitro experiments is very challenging. Therefore, mathematical models have the potential to give a significant contribution to the advancement of our understanding of the mechanisms underlying water and chemical species transport across epithelial layers. In particular, mathematical models can help isolate the role of specific effects and can also provide suggestions for experiments to be performed. Clearly, one of the fundamental ingredients to inform mathematical models of water transport across epithelia is a proper description of ion channels on the cell membranes and their response to external stimuli and interventions, such as therapeutics. Further experimental research in this area is of utmost importance for enhancing the quantitative understanding of the ocular water transport and its physiology, and would be also indispensable in the parameterization and validation of models.

In spite of the importance that mathematical models can have in this field, relatively few studies have been conducted and are far from comprehensive, and hence there is an enormous potential for future modeling activity. An ingredient that has largely been disregarded so far is the importance of metabolism on the functioning of ocular epithelial layers and, in particular, the role of lactate (with the notable excep-

tion of Leung et al.³³ and Cheng and Pinsky³⁴). For instance, a lactate cotransporter is thought to contribute to water transport across the RPE and the corneal endothelium, where significant metabolically induced lactate gradients exist.⁴⁶

Finally, we would like to highlight that these models form a small cog in a large machine, the human eye, which encompasses scales from the molecular to the organ. Representing such detail is leading to evermore complex studies and presents both novel opportunities –in particular, in moving towards a virtual eye– and novel challenges –in particular validation, reproducibility, sustainability, and modularity– for ocular modeling research.

Declarations

Ethics approval and consent to participate

None required, as this is a review.

Competing interests

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