The Balance of Fluid and Osmotic Pressures across Active Biological Membranes with Application to the Corneal Endothelium

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Abstract

The movement of fluid and solutes across biological membranes facilitates the transport of nutrients for living organisms and maintains the fluid and osmotic pressures in biological systems. Understanding the pressure balances across membranes is crucial for studying fluid and electrolyte homeostasis in living systems, and is an area of active research. In this study, a set of enhanced Kedem-Katchalsky (KK) equations is proposed to describe fluxes of water and solutes across biological membranes, and is applied to analyze the relationship between fluid and osmotic pressures, accounting for active transport mechanisms that propel substances against their concentration gradients and for fixed charges that alter ionic distributions in separated environments. The equilibrium analysis demonstrates that the proposed theory recovers the Donnan osmotic pressure and can predict the correct fluid pressure difference across membranes, a result which cannot be achieved by existing KK theories due to the neglect of fixed charges. The steady-state analysis on active membranes suggests a new pressure mechanism which balances the fluid pressure together with the osmotic pressure. The source of this pressure arises from active ionic fluxes and from interactions between solvent and solutes in membrane transport. We apply the proposed theory to study the transendothelial fluid pressure in the *in vivo* cornea, which is a crucial factor maintaining the hydration and transparency of the tissue. The results show the importance of the proposed pressure mechanism in mediating stromal fluid pressure and provide a new interpretation of the pressure modulation mechanism in the *in vivo* cornea.

Introduction

The exchange of fluid and solutes across biological membranes facilitates the transport of substances needed for living organisms to maintain their metabolic activities, and regulates pressure balances across bounding membranes to maintain the structural integrity of biological systems. The movement of these substances is controlled by both passive and active transport processes. Passive transport mechanisms drive water or solutes to move down their concentration gradients without need of energy input, whereas active transport mechanisms propels

solutes to move against their concentration gradients at the cost of energy input from metabolic reactions. The interplay between the two mechanisms determines the fluid hydrostatic pressure and osmotic pressure differences across biological membranes, which are important characteristics for biological systems. For example, at the organ level, fluid pressure mediates fluid transport between capillaries and tissues, and facilitates the diffusion of nutrients between the two compartments [1]. At the cellular level, fluid pressure interacts with osmotic pressure to regulate cell volumes at normal state [2, 3] and to drive its shape changes during processes such as protrusion and blebbing [4, 5]. A disturbance of the pressure regulation mechanisms can lead to swelling or shrinking of cells and tissues. A quantitative understanding of fluid and osmotic pressures in living organisms is crucial for studying biological mechanisms such as cell volume regulation and interstitial fluid homeostasis, and is under investigation for various biological systems, e.g. [2, 3, 6, 7]. The goal of the present work is to develop a mathematical description of water and solute transport across membranes and apply it to study the pressure balance conditions in biological systems, characterizing passive and active transport mechanisms and other biological features.

Cells and connective tissues contain a significant concentration of negatively charged molecules (proteins, organic phosphates or sulphated proteoglycans) to which the bounding membrane is impermeable [3, 8]. The presence of fixed charges attracts counterions and produces an osmolarity that is higher in the internal compartment than in the external compartment, which is separated from the internal compartment by the membrane. As a result, an osmotic pressure gradient is developed which drives water to flow into the interior compartment and thereby cause swelling. In many physiological systems, the above noted swelling tendency is counterbalanced by active ionic transport processes that are located in bounding membranes and which produce outward fluxes of ions that can modulate the osmotic pressure induced by fixed charges [3]. In animal cells, the maintenance of cell volumes relies heavily on the pressure balance mediated by active ionic transport processes and intracellular fixed charges [2, 3].

In order to describe transport phenomena in such biological systems, nonequilibrium thermodynamics has been employed to characterize the relationship between fluid and ionic fluxes and their generalized driving forces [9–11], with the latter originating in the gradient of the electrochemical potential and affinity of metabolic reactions. The theory is predicated on the existence of a dissipation function which describes the total change of system entropy. For near-equilibrium systems, in which the rate of free energy dissipation is small (i.e. the system is "is not too far" from equilibrium), linear relationships between fluxes and the driving forces can be assumed [9, 11]. The classical Kedem-Katchalsky (KK) theory [12] was developed along these lines. KK theory takes account of interactions between solvent and non-ionic solutes, and results in a set of phenomenological membrane coefficients that are readily evaluated experimentally. This theory provides the theoretical foundations for analyzing fluid flow and solute fluxes of various membrane systems.

Because the original KK theory was limited to passive transport in nonelectrolytes, numerous extensions and modifications have been proposed to include additional biophysical effects. Kedem and Katchalsky [13] extended the original KK equations to study the transport of a binary electrolyte through charged membranes. In addition to consideration of water flow and ionic fluxes, the effect of electric currents has been incorporated into KK theory to describe electroosmosis phenomena. Hoshiko and Lindley [14] integrated active ionic transport mechanisms into the KK equations for single salt and bi-ionic systems. This work developed a convenient approach to describe active movement of ions in a membrane system without the need to know the underlying detailed molecular mechanisms. Kargol [15] proposed a set of modified KK equations that takes into account the effect of boundary layers on the passive transport across a membrane. The modified theory is shown to give better prediction for glucose flux

across a nephrophane membrane. More recently, Li [16] derived a new set of KK equations for the transport of multiple ionic species across membranes. This work derived a new volume flux formulation which includes an additional driving force that originates from the transmembrane electrostatic potential difference.

While these developments of the KK theory have covered a wide range of biophysical conditions, the effect of fixed charges has been neglected; KK theories to date have only considered mobile ions and employed the electroneutrality assumption (i.e. $\Sigma_i z_i C_i = 0$). In the current study, we evaluate the influence of fixed charges on fluid and solutes transport across biological membranes. First we show that due to neglect of fixed charges, existing KK theories, including [12, 16], predict zero fluid pressure difference across membranes at thermodynamic equilibrium. We then employ linear nonequilibrium thermodynamics and propose a set of enhanced KK equations considering: 1) the presence of fixed charges on one side of a membrane, 2) transport of both ionic and non-ionic species and 3) active ionic transport mechanisms which move solutes from a lower to a higher concentration region. The proposed theory is capable of recovering the Donnan equilibrium and predicting the correct fluid pressure that is required to balance the Donnan osmotic pressure at equilibrium state. The analysis explains the swelling tendency of a charged electrolyte gel regardless of the presence of bounding layers.

In addition to illustrating the fixed charge effect, we apply the proposed KK equations to study the water transport across active biological membranes, which was believed to be governed by the balance of osmotic pressure and fluid pressure [3]. Our analysis identifies an additional pressure mechanism that originates from active fluxes and from interactions between water and solutes in membrane transport processes. This pressure force competes with the osmotic pressure on balancing the fluid pressure, and the new pressure balance condition implies that the values of the water potential on the two sides of separating membranes will not be equal in order to maintain the steady state of biological systems. To illustrate the importance of this new pressure mechanism, we apply the enhanced KK equations to quantify the transendothelial fluid pressure in the *in vivo* cornea, in which active transport mechanisms play crucial roles in regulating the fluid transport across the corneal endothelial layer. The results show that the additional pressure mechanism has a significant impact on influencing the fluid pressure.

Limitations of existing Kedem-Katchalsky theories

In this section we describe a limitation of existing KK theories [12, 16] which are unable to recover the Donnan equilibrium when fixed charges exist on one side of the membrane. Consider a biological membrane that separates two polyelectrolyte solutions with fluid pressure P and P_0, solute concentrations C_i and $C_i^0 (i = 1, ..., N)$, where N denotes the number of species, and electrostatic potential φ and φ_0. We denote one side of the membrane as "inside" and the other as "outside" (see Fig 1) and assume the inside electrolyte solution contains large molecules that carry fixed charges with concentration C_f and valence value z_f. The fixed charges are assumed to be "trapped" in the inside solution and the biological membrane is assumed to be impermeable to large molecules [3]. Both solvent and solutes are considered to have finite permeabilities through the membrane (i.e. the membrane is leaky). The classical KK equations [12] describe the volume flux J_V and solute flux $J_i(i = 1, ..., N)$ between the two solutions as follows:

$$J_V = L_p \left(\Delta P - \sum_k \sigma_k RT \Delta C_k \right) \qquad (1)$$

$$J_i = (1 - \sigma_i) J_V \overline{C}_i + \omega_i RT \Delta C_i \qquad (2)$$
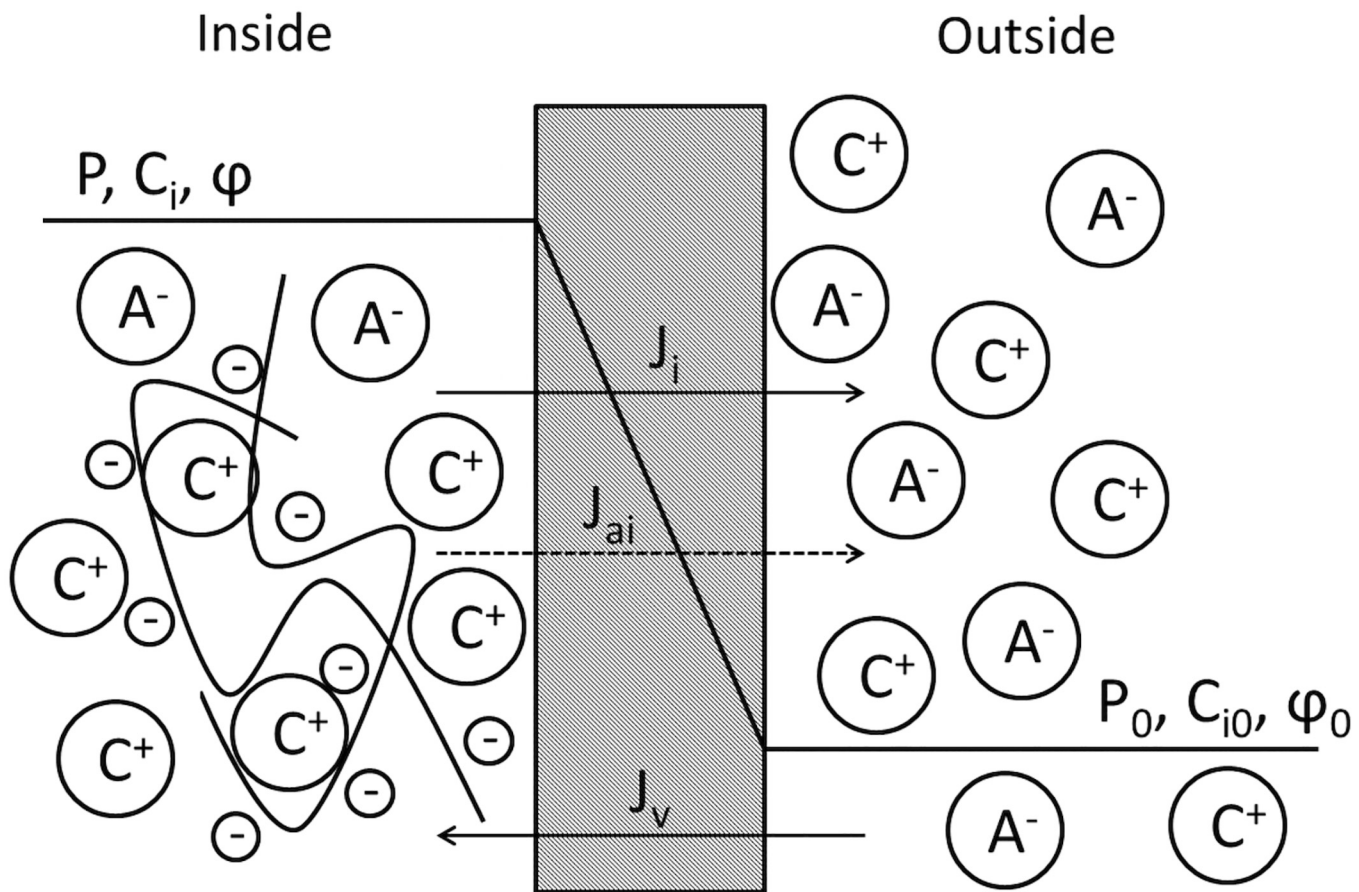
Inside

Outside

Fig 1. Illustration of a biological membrane that separates two electrolyte solutions, with the one designated as "inside" containing fixed charges that are associated with large molecules.

where L_p is the hydraulic conductivity, σ_i and ω_i are the reflection coefficient and permeability for species i, respectively, and ΔP and ΔC_i are the fluid pressure difference and ionic concentration difference across the membrane, respectively. $\overline{C}_i$ denotes the mean ionic concentration, and can be simplified as the arithmetic mean between C_i and C_i^0 (i.e. $\overline{C}_i = (C_i + C_i^0)/2$). Consider the equilibrium condition in which no fluid flow and no ionic fluxes exist across the membrane, i.e. $J_V = J_i = 0$, Eqs (1, 2) immediately give

$$\Delta C_i = 0 \tag{3}$$

$$\Delta P = 0 \tag{4}$$

which suggests that at equilibrium, ionic concentrations will be balanced and there will be no fluid pressure difference across the membrane. This conclusion is apparently contradicted by the well-known Donnan equilibrium where fixed charges induce imbalance of ionic concentrations and develop an osmotic pressure gradient between the inside and outside environments [17]. This limitation of Eqs (1, 2) is attributed to the fact that they were developed for transport of non-ionic species [12]. Li [16] derived an extended set of KK equations which incorporate

the electrostatic potential difference between separated electrolyte solutions,

$$J_V = L_p\left(\Delta P - \sum_k \sigma_k\left(RT\Delta C_k + z_k F\overline{C}_k \Delta\varphi\right)\right) \tag{5}$$

$$J_i = (1 - \sigma_i)J_V\overline{C}_i + \omega_i\left(RT\Delta C_i + z_i\overline{C}_i F\Delta\varphi\right) \tag{6}$$

The above equations are still unable to recover the fluid pressure difference corresponding to Donnan equilibrium. At the equilibrium state (with $J_V = J_i = 0$), Eqs (5, 6) imply zero pressure difference and nonzero ionic concentrations difference, which suggests a nonphysical situation. Regardless of the ionic concentrations difference across the membrane due to the fixed charges, no fluid pressure difference is needed to balance the resulting osmotic pressure difference.

The inconsistency between Eqs (5, 6) and the Donnan theory is associated with the neglect of the fixed charges. Presently, the only KK theory that incorporates the fixed charge effect is the one proposed by Hodson and Earlam [18] considering binary solutions:

$$J_V = L_p((\Delta P - \sigma\Delta\Pi) - (1 - \sigma)\Delta\gamma) \tag{7}$$

$$J_i = (1 - \sigma)J_V C_0 + \frac{\omega}{\sigma}(\Delta P - \Delta\gamma) \tag{8}$$

where σ and ω are the membrane transport coefficients, C_0 is the binary electrolyte concentration of the outside solution, $\Delta\Pi = RT(C_+ + C_- - 2C_0)$ is the osmotic pressure, and $\Delta\gamma = RTC_f^2/4C_0$ is called the gel pressure and is a function of fixed charge concentration. Interestingly, applying the zero flux conditions to Eqs (7, 8) gives $\Delta P = \Delta\gamma = \Delta\Pi$, although the theory is only feasible for binary solutions and no active transport is considered. In this study, we employ linear non-equilibrium thermodynamics and derive a modified form of the KK equations for a polyelectrolyte solution with fixed charges. We then further extend the theory to include active ion transport mechanisms.

Modified KK equations for passive transport

Consider a leaky membrane that separates two electrolyte solutions as described above. The movement of solvent and solutes across the membrane can be characterized by a set of fluxes and conjugate forces according to nonequilibrium thermodynamics [11]. The identifications of these quantities are based on the statement of dissipation function Φ, which describes the rate that the free energy is dissipated during transport. Its mathematical formulation is given as [9, 10]

$$\Phi = J_w X_w + \sum_{i=1}^{N} J_i X_i \tag{9}$$

where J_w and J_i ($i = 1, \ldots, N$) denote fluid flow and solute fluxes, respectively, and X_w and X_i denote the corresponding conjugate forces for fluid and solutes. In their simplest form, X_w and X_i can be written as the electrochemical potential difference across the membrane [10, 12], i.e.

$$X_w = \Delta\mu_w \tag{10}$$

$$X_i = \Delta\mu_i \tag{11}$$

where μ_w and μ_i are the electrochemical potential for fluid and solute species i, respectively. In order to derive the mathematical forms for $\Delta\mu_w$ and $\Delta\mu_i$, we first recall the electrochemical

potential for water and ions as:

$$\mu_w = v_w \left(P - RT \sum_k C_k \right) \tag{12}$$

$$\mu_i = v_i P + RT \ln C_i + z_i F \varphi \tag{13}$$

where v_w and v_i in m^3/mol are the partial volume of solvent and solutes, respectively, z_i is the valence number for species i, R, T and F are gas constant, temperature and Faraday constant, respectively. The linearized forms for $\Delta\mu_w$ and $\Delta\mu_i$ are then given as

$$\Delta\mu_w = v_w \left(\Delta P - RT \sum_k \Delta C_k \right) \tag{14}$$

$$\Delta\mu_i = v_i \Delta P + RT \frac{\Delta C_i}{\overline{C}_i} + z_i F \Delta\varphi \tag{15}$$

where $\overline{C}_i = (C_i + C_i^0)/2$ is the average ionic concentration through the membrane.

Following a common convention [12, 16], we rewrite the dissipation function in terms of a new set of forces and fluxes that are easier to assess experimentally

$$\begin{aligned} \Phi &= J_w \Delta\mu_w + \sum_i J_i \Delta\mu_i \\ &= \left(v_w J_w + \sum_k v_k J_k \right) \Delta\tilde{P} + \sum_i \left(\frac{J_i}{\overline{C}_i} - \frac{J_w}{C_w} \right) \Delta\tilde{\Pi}_i \\ &= J_V \Delta\tilde{P} + \sum_i J_{Di} \Delta\tilde{\Pi}_i \end{aligned} \tag{16}$$

where C_w is the solvent (water) concentration, $J_V = v_w J_w + \sum_k v_k J_k$ is the volume flux across the membrane, and $J_{Di} = J_i/\overline{C}_i - J_w/C_w$ is the exchange flux for species i and can be interpreted as the relative velocity of the solute versus the solvent. The corresponding conjugate forces $\Delta\tilde{P}$ and $\Delta\tilde{\Pi}_i$ are given as

$$\Delta\tilde{P} = \left(C_w v_w + \sum_k C_k v_k \right) \Delta P + RT(1 - C_w v_w) \sum_k \Delta C_k + RT \sum_k z_k F \overline{C}_k \Delta\varphi \tag{17}$$

$$\Delta\tilde{\Pi}_i = RT \Delta C_i + z_i F \overline{C}_i \Delta\varphi - \overline{C}_i v_i \sum_k z_k F \overline{C}_k \Delta\varphi \tag{18}$$

Note that the sum of all volume fractions is unity, i.e.

$$C_w v_w + \sum_k C_k v_k = 1 \tag{19}$$

for dilute solutions, the solvent (water) volume fraction is dominant over that of solutes, and

therefore is close to one, i.e. $C_w v_w \approx 1$. Eqs ([17](#)) and ([18](#)) can then be simplified as

$$\Delta \tilde{P} = \Delta P + RT \sum_k z_k F \overline{C}_k \Delta \varphi \tag{20}$$

$$\Delta \tilde{\Pi}_i = RT \Delta C_i + z_i F \overline{C}_i \Delta \varphi \tag{21}$$

where [Eq (20)](#) describes the effects of fluid pressure and electrostatic potential on the volume flux, and is a more general expression compared to that given in [16], which eliminates the second term using an electroneutrality condition of the form $\sum_k z_k C_k = 0$ (i.e. neglecting the fixed charges). The new [formulation (20)](#) will lead to a set of modified KK equations which predict the correct fluid pressure in Donnan equilibrium.

Employing linear nonequilibrium thermodynamics [9, 10], the volume flux J_V and the exchange flux J_{Di} are assumed to be linear functions of the conjugate forces $\Delta \tilde{P}$ and $\Delta \tilde{\Pi}_i$,

$$J_V = L_{VV} \Delta \tilde{P} + \sum_k L_{Vk} \Delta \tilde{\Pi}_k \tag{22}$$

$$J_{Di} = L_{iV} \Delta \tilde{P} + \sum_k L_{Dik} \Delta \tilde{\Pi}_k \tag{23}$$

where $L_{Vk} = L_{kV}$, $k = 1, \ldots, N$ and $L_{Dij} = L_{Dji}$, $i, j = 1, \ldots, N$, satisfying the Onsager reciprocal relation Schultz1980. Rewriting the above equations as

$$J_V = L_p \Delta \tilde{P} - L_p \sum_k \sigma_k \Delta \tilde{\Pi}_k \tag{24}$$

$$J_{Di} = -L_p \sigma_i \Delta \tilde{P} + \sum_k L_{Dik} \Delta \tilde{\Pi}_k \tag{25}$$

where L_p and σ_k are the hydraulic conductivity coefficients and reflection coefficient that have the following formal definition:

$$L_p \equiv \left(\frac{J_V}{\Delta \tilde{P}} \right)_{\Delta \tilde{\Pi}_k = 0} = L_{VV} \tag{26}$$

$$\sigma_i \equiv -\frac{L_{Vi}}{L_{VV}} \tag{27}$$

Assuming a dilute solution, the solute flux J_i can be expressed as [10]:

$$\begin{aligned} J_i &= (J_V + J_{Di}) \overline{C}_i \\ &= (1 - \sigma_i) J_V \overline{C}_i + \sum_k \omega_{ik} \Delta \tilde{\Pi}_k \end{aligned} \tag{28}$$

where $\omega_{ik} = \overline{C}_i (L_{Dik} - L_p \sigma_i \sigma_k)$ denotes the permeability coefficients. Ignoring the interactions between ionic fluxes (i.e. $\omega_{ik} = 0$ for $i \neq k$) and denote $\omega_i = \omega_{ii}$,

$$J_i = (1 - \sigma_i) J_V \overline{C}_i + \omega_i \Delta \tilde{\Pi}_i \tag{29}$$

where ω_i has the following formal definition

$$\omega_i \equiv \left(\frac{J_i}{\Delta \tilde{\Pi}_i} \right)_{J_V=0} \tag{30}$$

In summary, the volume flux J_V and solute flux J_i can be written as a linear combination of ΔP, ΔC_i and $\Delta \varphi$ by substituting Eqs (20, 21) into Eqs (24, 29):

$$J_V = L_p \left(\Delta P - \sum_k \left(\sigma_k RT \Delta C_k - (1 - \sigma_k) z_k F \overline{C}_k \Delta \varphi \right) \right) \tag{31}$$

$$J_i = (1 - \sigma_i) J_V \overline{C}_i + \omega_i \left(RT \Delta C_i + z_i \overline{C}_i F \Delta \varphi \right) \tag{32}$$

It should be noted that the volume flux expression Eq (31) differs from Eq (5) by the addition of the term $-\sum_k z_k F \overline{C}_k \Delta \phi$, which is essentially zero in Li [16] due to neglect of the fixed charge concentration. The effect of this correction term will be discussed in the following section.

Application to equilibrium conditions: recovery of Donnan equilibrium

In this section the modified KK Eqs (31) and (32) are used to study the Donnan equilibrium in which unequal distributions of ionic concentration and fluid pressure are developed between two ionic solutions separated by a membrane [17]. At thermodynamic equilibrium, no macroscopic flow of fluid or solutes occurs between the two solutions, i.e. $J_V = J_i = 0$, and the dissipation function is zero (see Eq (9)). Applying the KK theory to study this classical condition provides the baseline for the study of the effects of active fluxes. We show that the new theory recovers the Donnan osmotic pressure, and predicts the correct fluid pressure that is required to balance the osmotic pressure. The predicted pressure quantities do not depend on membrane transport properties, indicating that the Donnan equilibrium will be satisfied regardless of the presence of a biological membrane [18].

At equilibrium, the zero flux conditions $J_V = 0$ and $J_i = 0$ requires

$$\omega_i \left(RT \Delta C_i + z_i \overline{C}_i F \Delta \varphi \right) = 0$$

which implies

$$-(1 - \sigma_i) z_i \overline{C}_i F \Delta \varphi = (1 - \sigma_i) RT \Delta C_i \tag{33}$$

The equilibrium fluid pressure difference ΔP, derived from $J_V = 0$, is then

$$\Delta P = \sum_k \left(\sigma_k RT \Delta C_k - (1 - \sigma_k) z_k F \overline{C}_k \Delta \varphi \right)$$

$$= \sum_k \sigma_k RT \Delta C_k + (1 - \sigma_k) RT \Delta C_k \tag{34}$$

$$= RT \sum_k \Delta C_k$$

From Eq (34) it can be seen that $\Delta P = RT \sum_k \Delta C_k = \Delta \Pi$ is satisfied independently of the membrane properties (σ_i, ω_i), indicating that regardless of whether the membrane is nearly

semipermeable ($\sigma_k \rightarrow 1$) or completely freely permeable ($\sigma_k \rightarrow 0$), the equilibrium fluid pressure difference always equals the osmotic pressure difference across the membrane.

Next we derive the mathematical formulation for ΔC_k and show that ΔP equals the Donnan osmotic pressure. Consider the separated solutions shown in Fig 1 as binary solutions, and assume the valence value of the fixed charges as $z_f = -1$. The sign convention is given as follows: net fluxes of fluid and solutes from the inside to the outside solution are taken as positive, and $\Delta(\cdot)$ is calculated by the quantity in the inside minus that in the outside. The equilibrium conditions $J_+ = J_- = 0$ then give:

$$RT(C_+ - C_0) + \frac{C_0 + C_+}{2} F\Delta\varphi = 0 \tag{35}$$

$$RT(C_- - C_0) - \frac{C_0 + C_-}{2} F\Delta\varphi = 0 \tag{36}$$

where $C_0 = C_+^0 = C_-^0$ is the bath concentration. The above equations result in

$$C_+ C_- = C_0^2 \tag{37}$$

which is the Donnan equilibrium condition [17]. Using the electroneutrality condition for the electrolyte, including the fixed charge,

$$C_+ = C_- + C_f \tag{38}$$

the cation and anion concentrations can be solved from Eqs (37, 38), and ΔP is given as:

$$\begin{aligned} \Delta P &= RT(C_+ + C_- - 2C_0) \\ &= 2RTC_0 \left(\sqrt{\frac{C_f^2}{4C_0^2} + 1} - 1 \right) \end{aligned} \tag{39}$$

which recovers the Donnan osmotic pressure [19]. The estimation of ΔP has important implications for membrane systems, since the fluid pressure difference corresponds to a mechanical force difference applied at the membrane. Positive ΔP indicates an expansion (swelling) tendency for inside of the membrane, and vice versa. It governs the volume of the interior compartment from bounding membranes, for example in cells [2], in vesicles [20] and in the human cornea [21].

Extension to include the effect of active ionic transport

Active transport across cell membranes enables solute movement against their concentration gradient and is one of the major factors for keeping homeostasis within the body. It is divided into two types, according to the source of energy used, called primary active transport and secondary active transport. In the former category, energy is directly provided by the breakdown of adenosine triphosphate (ATP). In the latter category, energy is derived indirectly from energy stored in the form of ionic concentration differences between the two sides of a membrane. Directly modeling active mechanisms requires identifications of the reaction kinetics, which is only known for a few processes [22, 23]. Alternatively, active mechanisms can be incorporated into the nonequilibrium thermodynamic description of fluid and ionic transport by introducing the affinity of the driving metabolic reaction and its conjugate flux, the rate of reaction per unit membrane area [10, 14] (see details in S1 Appendix A). In the simplest form, the active ionic flux is treated as an independent term that is additive to the passive solute flux

equation Eq (32). The net solute flux equation can then be written as

$$J_i = (1 - \sigma_i)J_V\overline{C}_i + \omega_i(RT\Delta C_i + z_i\overline{C}_iF\Delta\varphi) + J_{ai} \tag{40}$$

It is noted that Eq (40) recovers the solute flux equation as used in [21, 24, 25] to describe the effect of active ionic flux without considering the underlying molecular mechanisms.

Similar to the case of thermodynamic equilibrium, we apply the extended KK Eqs (31, 40) to study the zero flux conditions for J_V and J_i. As active transport mechanisms are presented and consume energy, the zero flux conditions correspond to a non-equilibrium steady state [26]. In this case, the fluid pressure difference ΔP across the membrane is derived by first observing from Eq (31) with $J_V = 0$ and Eq (32) with $J_i = 0$, that

$$\omega_i(RT\Delta C_i + z_i\overline{C}_iF\Delta\varphi) + J_{ai} = 0$$

which implies

$$-(1 - \sigma_i)z_i\overline{C}_iF\Delta\varphi = (1 - \sigma_i)RT\Delta C_i + \frac{1 - \sigma_i}{\omega_i}J_{ai} \tag{41}$$

Now $J_V = 0$ implies

$$\Delta P = \sum_i \left(RT\Delta C_i + \frac{1 - \sigma_i}{\omega_i}J_{ai} \right) = \Delta\Pi + P_\sigma \tag{42}$$

where $\Delta\Pi = RT\sum_i \Delta C_i$ denotes the osmotic pressure and $P_\sigma = \sum_i (1-\sigma_i)J_{ai}/\omega_i$ denotes an additional pressure force originating from the active ionic transport when the membrane is leaky ($\sigma_i < 1$).

The effect of P_σ on the fluid pressure difference ΔP may be studied by considering two binary electrolyte solutions, as described above, with an active anion flux J_{a-}. For convenience, we denote $J_a = J_{a-}$, $\omega = \omega_{Cl^-}$ and $\sigma = \sigma_-$. Eqs (35, 36) then become

$$RT(C_+ - C_0) + \frac{C_0 + C_+}{2}F\Delta\varphi = 0 \tag{43}$$

$$RT(C_- - C_0) - \frac{C_0 + C_-}{2}F\Delta\varphi + \frac{J_a}{\omega} = 0 \tag{44}$$

Solving Eqs (43), (44) and (38), the cation and anion concentrations are given as

$$C_+ = \frac{C_f}{2} - \frac{J_a}{4\omega RT} + \sqrt{\left(\frac{C_f}{2} - \frac{J_a}{4\omega RT}\right)^2 - \frac{J_a}{2\omega RT}C_0 + C_0^2} \tag{45}$$

$$C_- = -\frac{C_f}{2} - \frac{J_a}{4\omega RT} + \sqrt{\left(\frac{C_f}{2} - \frac{J_a}{4\omega RT}\right)^2 - \frac{J_a}{2\omega RT}C_0 + C_0^2} \tag{46}$$

and the steady-state ΔP is then obtained from Eq (42) as:

$$\Delta P = 2RTC_0\left(\sqrt{\left(\frac{C_f}{2C_0} - \frac{J_a}{4\omega RTC_0}\right)^2 - \frac{J_a}{2\omega RTC_0} + 1} - \left(\frac{\sigma}{2} - \frac{1}{4}\right)\frac{J_a}{\omega RTC_0} - 1 \right) \tag{47}$$

It can be seen that there are three free parameters controlling ΔP: the fixed charge concentration C_f, the active ionic flux divided by the membrane permeability J_a/ω and the reflection coefficient σ. The effects of these quantities on ΔP are discussed as follows.
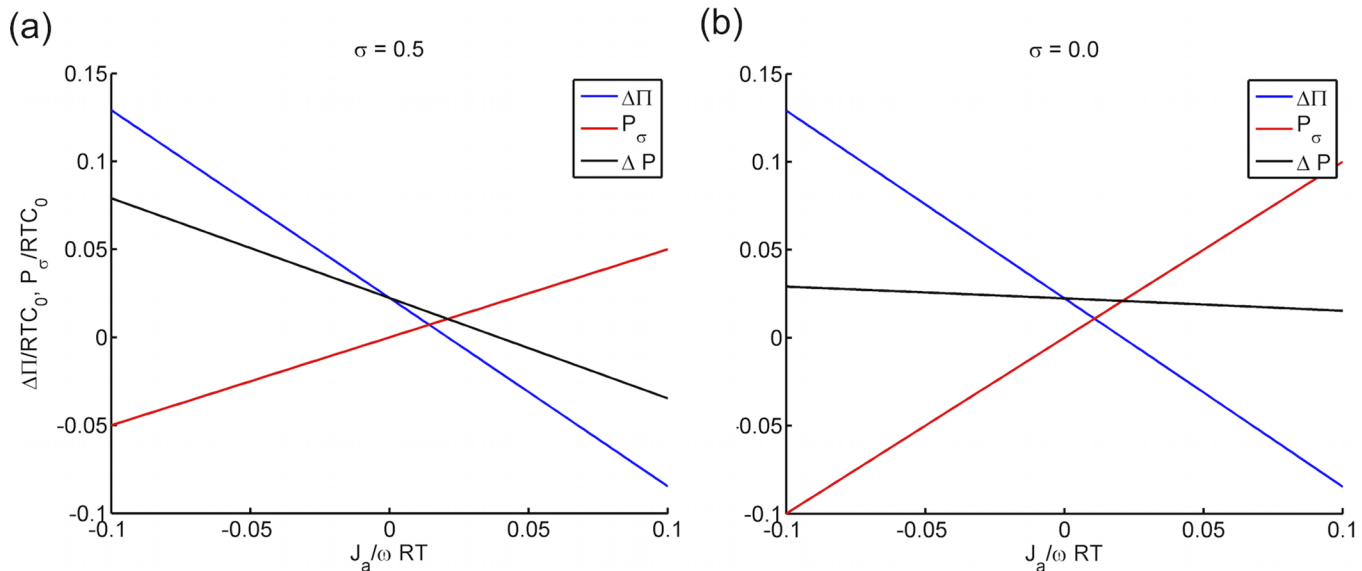
Fig 2. The predicted uid pressure difference ΔP, osmotic pressure difference ΔΠ and the additional pressure force P_σ against active ionic flux J_a for a) a leaky membrane with $\sigma = 0.5$ and b) a freely permeable membrane ($\sigma = 0.0$).

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The pressure, fixed charge density and ionic fluxes are expressed in dimensionless forms as $\Delta P/RTC_0$, C_f/C_0 and $J_a/\omega RTC_0$, respectively. The effect of active ionic flux on P_σ, $\Delta\Pi$ and ΔP are summarized in Fig 2 over the range of $J_a/\omega RTC_0 \in [-0.1, 0.1]$ at a fixed charge density $C_f/C_0 = 0.3$. Two values of σ, one representing a leaky membrane ($\sigma = 0.5$) and the other representing a freely permeable membrane ($\sigma = 0.0$), have been selected to illustrate the effect of P_σ on ΔP. An essentially linear relationship is observed between the active ionic flux and the three pressure estimations. The osmotic pressure $\Delta\Pi$ is reduced when the active ionic flux is directed from inside to outside as described by positive J_a. On the contrary, predicted P_σ increases with J_a. The fluid pressure ΔP, which results from the two competing terms, decreases with J_a, indicating that the active ionic transport has a stronger effect on $\Delta\Pi$ than on P_σ. Notably, the influence of P_σ on the fluid pressure difference is significant, especially when the membrane is more permeable (as described by small σ) to the active species. For $\sigma = 0.5$, the ratio between P_σ and $\Delta\Pi$ is about 0.6 at $J_a/\omega RTC_0 = 0.1$ (see Fig 2a). For a membrane that is freely permeable ($\sigma = 0.0$), the corresponding ratio increases to 1.2, resulting in a fluid pressure curve that is nearly unaffected by J_a (see Fig 2b). If the membrane is nearly semipermeable ($\sigma \rightarrow 1.0$), P_σ is zero and has no effect on ΔP. The results in Fig 2 show that the pressure force P_σ plays a significant role in countering the active ionic transport effect on the fluid pressure.

Application to endothelial transport in the *in vivo* cornea

In this section an example application of Eqs (31, 40) is presented to study the endothelial transport process of the *in vivo* human cornea (see Fig 3). The exchange of fluid and ions across the endothelium controls the level of corneal hydration, which is a crucial factor for maintaining the transparency of the tissue [21, 24]. Fixed charges are associated with sulphated proteoglycans in the stroma (the bulk layer of the tissue), and generate osmotic pressure by Donnan effect [8, 27]. The active ionic transport processes located in the endothelium reduce the osmotic pressure by pumping ions out from the tissue. Furthermore, metabolic reactions take place in the *in vivo* cornea, rendering nonzero transendothelial fluxes for metabolic species (glucose, bicarbonate and lactate ions) [25, 28, 29].
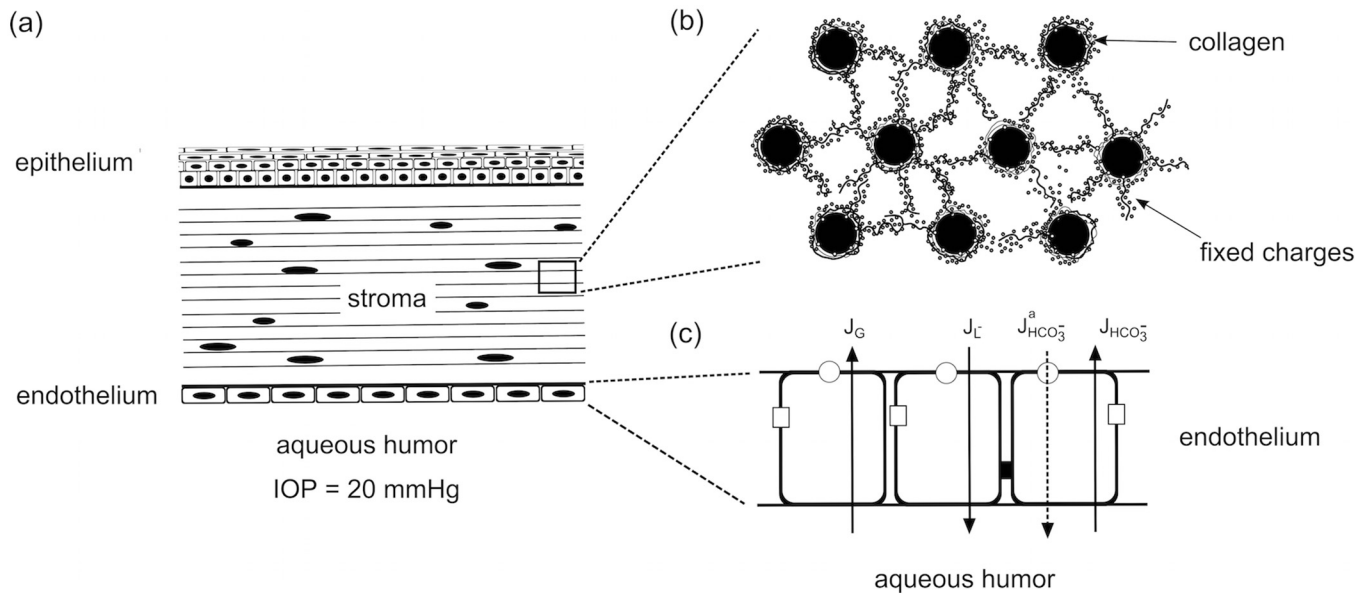
Fig 3. a) The cornea has three principal layers, namely the epithelium, stroma and endothelium. b) The corneal stroma is composed by collagen fibers (lamellae) packed through thickness, with keratocyte cells interspersed between adjacent lamellae. Fixed charges are associated with sulphated proteoglycans that are attached to the collagen fibrils, which form a lamella by assembling as parallel arrays following the direction of the lamella. The fixed charges give rise to a swelling tendency of the cornea [8] by the Donnan effect [17]. c) The *in vivo* cornea modulates the osmotic pressure by actively pumping ionic solutes (primarily bicarbonate) from stroma to aqueous humor [31]. In addition, glucose transports from aqueous humor to stroma for the metabolic activities needed by corneal cells, and lactate ion transports in the opposite direction [25]. The underlying molecular mechanisms of corneal endothelial pumping are still under investigation [31].

At steady state, only the volume flux is zero, with the steady solute fluxes being nonzero in general. This is a more general situation than that discussed in the preceding section. We consider four ionic species, namely sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-) and lactate ions ($C_3H_5O_3^-$, denoted as L^-), and one non-ionic species (glucose, denoted as G) distributed on both sides of the endothelium. The concentrations of these species in the outside solution (aqueous humor) are listed in table 1. The reported reflection coefficients and solute permeabilities of corneal endothelium are given in table 2. Additional model input includes the net solute fluxes for metabolic species and the active bicarbonate flux [30], which were estimated by Leung et al. [25] and are summarized in table 3. The proposed KK theory now presents a set of nonlinear equations with seven unknowns (five solute concentration + fluid pressure

Table 1. Fields values in the aqueous humor [25].

Parameters	Value
$C_{Na^+}^0$ (mM)	146.55
$C_{Cl^-}^0$ (mM)	102.85
$C_{HCO_3^-}^0$ (mM)	36.00
$C_{L^-}^0$ (mM)	7.7
C_G^0 (mM)	6.9
P_0 (mmHg)	20.0
φ_0 (mV)	0.0

Table 2. Endothelial membrane properties [25].

Parameters	Value
σ_{Na^+}	0.45
σ_{Cl^-}	0.45
$\sigma_{HCO_3^-}$	0.38
σ_{L^-}	0.45
σ_G	0.45
$\omega_{Na^+} RT (10^{-5}$ cm/s)	8.0
$\omega_{Cl^-} RT (10^{-5}$ cm/s)	8.0
$\omega_{HCO_3^-} RT (10^{-5}$ cm/s)	8.0
$\omega_{L^-} RT (10^{-5}$ cm/s)	3.0
$\omega_G RT (10^{-5}$ cm/s)	8.0

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+ electrostatic potential), which are summarized as follows:

$$\Delta P - \sum_k \left(\sigma_k RT \Delta C_k - (1 - \sigma_k) z_k F \overline{C}_k \Delta\varphi \right) = 0 \tag{48}$$

$$\omega_i (RT \Delta C_i + z_i \overline{C}_i F \Delta\varphi) + J_{ai} = J_i \quad , i = Na^+, Cl^-, HCO_3^-, L^-, G, \tag{49}$$

$$\sum_k z_k C_k + z_f C_f = 0 \tag{50}$$

The fluid pressure difference ΔP is solved as

$$\Delta P = \sum_i \left(RT \Delta C_i + \frac{1 - \sigma_i}{\omega_i} (J_{ai} - J_i) \right) = \Delta\Pi + P_\sigma \tag{51}$$

where J_i denotes the net flow for species i and $P_\sigma = \Sigma_i (1 - \sigma_i)(J_{ai} - J_i)/\omega_i$. The solute concentration difference ΔC_i across the endothelium can be derived as (see details in S1 Appendix B):

$$\Delta C_i = -\frac{z_i F \Delta\varphi C_i^0 + \frac{J_{ai} - J_i}{\omega_i}}{RT + \frac{1}{2} z_i F \Delta\varphi} \tag{52}$$

where the electrostatic potential difference $\Delta\varphi$ is solved as (see in S1 Appendix B):

$$\Delta\varphi = \frac{-B + \sqrt{B^2 - 4AC}}{2A} \tag{53}$$

Table 3. Reported solute fluxes across corneal endothelium [25].

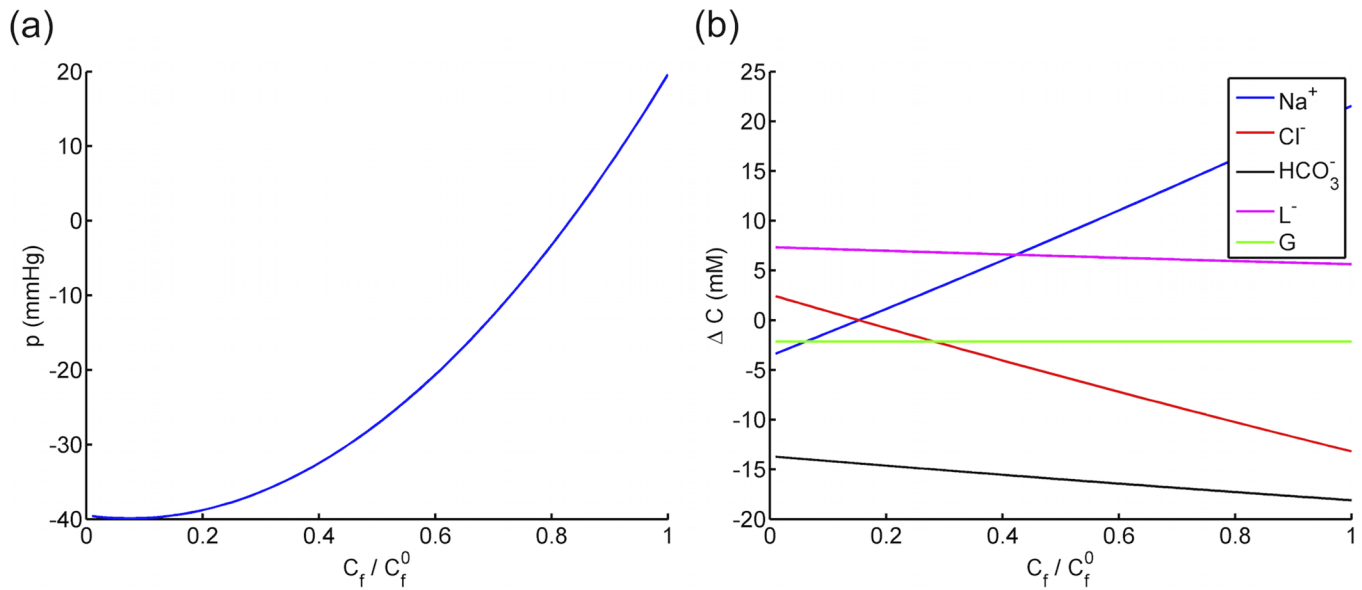| Parameters	Value
J_{Na^+}	0
J_{Cl^-}	0
$J_{HCO_3^-} (10^{-10} mol/cm^2 \cdot s)$	-2.12
$J_{HCO_3^-}^a (10^{-10} mol/cm^2 \cdot s)$	9.4
$J_{L^-} (10^{-10} mol/cm^2 \cdot s)$	2.12
$J_G (10^{-10} mol/cm^2 \cdot s)$	-1.71

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(a)

(b)

Fig 4. The effect of fixed charge concentration on a) the stromal uid pressure and b) the solute concentration differences across the corneal endothelium.

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where parameters A, B and C are

$$A = \frac{1}{4} z_f C_f \frac{F^2}{RT} \tag{54}$$

$$B = \frac{F}{RT}\left(RT\sum_k C_k^0 - \sum_k \frac{J_{ak} - J_k}{2\omega_k}\right) \tag{55}$$

$$C = -RT z_f C_f + \sum_k \frac{z_k J_{ak} - z_k J_k}{\omega_k} \tag{56}$$

The effects of fixed charges on fluid pressure and solute concentrations, given by Eqs (51, 52), are illustrated in Fig 4a and 4b in the range of $C_f \in [0, C_f^0]$, where $C_f^0 = 48$ mM is the measured fixed charge concentration in corneal stroma [8]. The stromal fluid pressure P is predicted to be highly sensitive to C_f, increasing from −39.5 to 19.6 mmHg (nearly 150% variation) as the fixed charge concentration varies from zero to C_f^0 (see Fig 4a). In the special case of $C_f = 0$ (no fixed charge), P is calculated to be −40 mmHg, which is comparable to the value reported by Leung et al. [25] based on the KK Eqs (5 and 6) (see table 4). The predicted sodium concentration difference ΔC_{Na^+}, given by Eq (52), shows the highest sensitivity to C_f among all the solutes (see

Table 4. Comparisons of the solute concentrations and stationary fluid pressure at stromal-endothelial interface of the cornea by the new KK equations and that by [25].

	C_{Na^+} (mM)	C_{Cl^-}	$C_{HCO_3^-}$	C_{L^-}	P (mmHg)
Leung et al. [25]	143.1	105.0	22.58	15.00	−40.5
New KK, $C_f = 0$	143.6	105.3	22.28	15.03	−39.5
New KK, $C_f = 48.0$ mM	168.5	89.67	17.91	13.33	19.6

doi:10.1371/journal.pone.0145422.t004

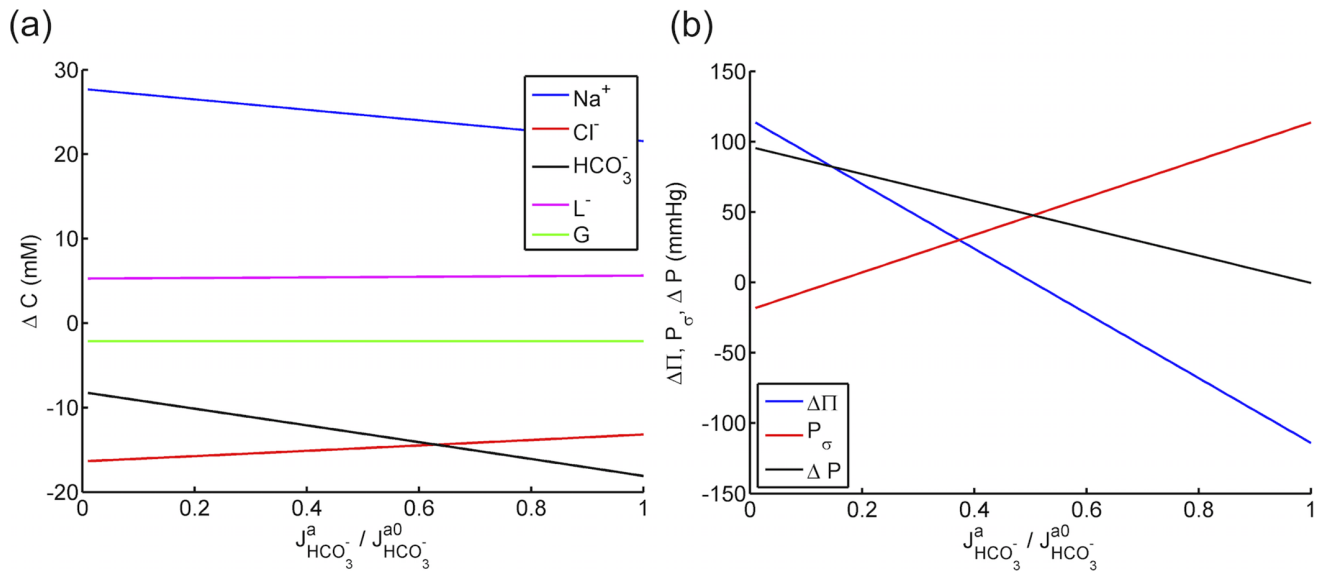(a)   (b)

Fig 5. a) The predicted solute concentration differences across corneal endothelium against active bicarbonate flux. b) The predicted uid pressure difference ΔP, osmotic pressure difference ΔΠ and the additional pressure force P_σ against active bicarbonate flux for the corneal endothelium.

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Fig 4b), varying from −2.2 to +22.0 mM as C_f increases from zero to C_f^0 (see also table 4). On the other hand, the predicted anion concentrations (chloride, bicarbonate and lactate ions) show an opposite trend by decreasing with C_f. The contrast between the curves of cation and anions is attributed to the Donnan effect, which requires accumulation of cations to compensate the concentration of negative fixed charges. As expected, the calculated glucose concentration is invariant with C_f since it is a neutral species. The calculated ionic concentrations at $C_f = 0$ are in close agreement with those obtained in Leung et al. [25] (see table 4).

The effect of active bicarbonate flux $J^a_{HCO_3^-}$ on solute concentrations is presented in Fig 5a in the range of $J^a_{HCO_3^-} \in [0, J^{a0}_{HCO_3^-}]$, where $J^{a0}_{HCO_3^-}$ denotes the reported value of bicarbonate pumping flux (see table 3). Predicted bicarbonate concentration shows the most sensitivity to $J^a_{HCO_3^-}$ where $\Delta C_{HCO_3^-}$ varies from −10 to −20 mM as the active bicarbonate flux increases from zero to $J^{a0}_{HCO_3^-}$. Interestingly, the sodium concentration difference ΔC_{Na^+} is also highly dependent on the bicarbonate pumping flux, reducing from 30 to about 22 mM. The chloride and lactate ion concentrations, on the other hand, increase mildly with $J^a_{HCO_3^-}$. The osmotic pressure, which is a measure of the total osmolarity including all the solutes, decreases as bicarbonate ions are pumped out from stroma to aqueous humor (see Fig 5b). The influence of $J^a_{HCO_3^-}$ on ΔP and P_σ is shown in Fig 5b. At the condition of no active transport ($J^a_{HCO_3^-} = 0$), fluid pressure difference ΔP is predicted to be approximately 100 mmHg, a nonphysiological value that will cause the tissue to swell severely and damage the endothelial membrane [21, 30]. This result confirms the necessity of the active transport mechanism to maintain the hydration of the tissue. When $J^a_{HCO_3^-} = J^{a0}_{HCO_3^-}$, ΔP is predicted to be zero which is a result of equal osmotic pressure ΔΠ and P_σ on the magnitude of 120 mmHg. The two curves of ΔΠ and P_σ have opposite signs of slope, indicating again that the two pressure mechanisms compete with each other in generating fluid pressure in the stroma: while ΔΠ is reduced due to the active transport of bicarbonate, P_σ provides a countering effect by increasing with $J^a_{HCO_3^-}$. The net effect of the two mechanisms, illustrated by the curve of ΔP, shows a decreasing trend with $J^a_{HCO_3^-}$ but with a smaller slope due to P_σ.

Discussion

The proposed KK Eqs (31, 40) present a general framework to describe coupled transport through biological membranes. This work may be viewed as an extension from Li [16], Hodson and Earlam [18] and Hoshiko and Lindley [14] by including the effect of fixed charges, multi-component solutes (both ionic and non-ionic species) and active transport mechanisms. While the derivations follow the standard practice of the KK theory [10], the key step is to take account of the fixed charge concentration. The theory resolves the difficulties of existing KK equations in predicting transmembrane fluid pressure for charged electrolyte solution. The recovery of the fluid pressure and osmotic pressure in Donnan equilibrium provides verification of the proposed theory, and explains the swelling tendency of tissues like cornea (with fixed charge inside) with or without the presence of a bounding membrane, a phenomena that has been observed experimentally and explained qualitatively [18].

As water transport is primarily passive in biological systems [32], conventional analysis treats water as being effectively in thermodynamic equilibrium [3], with the water potential difference across membrane assumed to be zero. In this case, the osmotic pressure difference $\Delta\Pi$ is balanced by the fluid pressure difference ΔP[21]. The analyses presented in the preceding two sections have shown that this may not be the case when active ionic transport processes are present. The generation of P_σ alters the pressure balance condition across membranes such that

$$\Delta P = \Delta\Pi + P_\sigma \Rightarrow \mu_w \neq \mu_w^0 \tag{57}$$

This implies that the values of the water potential across the separating membrane will not be equal. According to Eq (57), the fluid pressure difference is balanced by the combined effect from $\Delta\Pi$ and P_σ. Numerically, $\Delta\Pi$ and P_σ have opposite signs, indicating their competing effect in contributing to ΔP; if ions are pumped out to reduce the fluid pressure inside, the pressure P_σ will be positive and provide a countering effect, and vice versa. The quantitative analyses given in the preceding two sections show that P_σ has a significant impact on P when the membrane is leaky, indicated by a reflection coefficient less than unity. The significance of P_σ in biological system is also interesting to discuss. As active transport mechanisms take place across biological membranes to maintain the internal fluid pressure by removing ions from inside to the outside environment, the pressure force P_σ makes such processes less efficient—the membrane system is required to generate a stronger active flux to overcome P_σ, which costs more energy from the active transport mechanism.

Specifically for the cornea, the pressure Eq (57) suggests a new interpretation of the stromal fluid pressure, which is an important quantity in corneal biomechanics because it interacts with the stromal collagen and modulates the fluid content of the tissue [21]. Historical views on the transendothelial fluid balance have been predicated on the "pump-leak" hypothesis [30]. It assumes negative stromal fluid pressure, which contributes a fluid leak from the aqueous humor to the stroma, and negative osmotic pressure, which results in a fluid leak in the other direction. This balancing concept can be abstracted by $\Delta P = \Delta\Pi$, where the left hand side represents the source for the "leak" and the right hand side for the "pump." According to Eq (57), the balancing condition can be relaxed; due to the generation of P_σ, the stromal fluid pressure could be positive (see table 4) while the osmotic pressure is negative. This view of a positive stromal fluid pressure is corroborated by a recent structural analysis of the cornea [21], which needs further experimental verification.

It is clear from Eq (51) that P_σ is highly sensitive to reflection coefficient σ_i, which is a cross coefficient that provides a measure of the interaction between the flows of solute and solvent. Although the KK theory is strictly phenomenological [9], the physical interpretation of σ_i is

worth discussing. An alternative way of interpreting the membrane transport is to view the conjugate forces as a result of mechanical friction among solute (water) and solutes [33]. Accordingly, σ_i has been found to be governed by the solute permeability through the membrane and the friction between solvent and solute. It will be unity only if the membrane is strictly impermeable to solute species i. On the contrary, the more leaky the membrane is to solutes and the stronger the interactions between the solute and solvent phases, the smaller σ_i will be, which results in a larger P_σ as shown in the current analysis.

Extensions on the current work include two aspects. First, the new KK theory can be incorporated into continuum models for cells or tissues to describe the interactions between membrane transport and the bulk electrolyte inside. A multiphasic model for the living cornea that accounts for hydration, fixed charges and endothelial transport is under development. Second, it should be noted that the assumption of an independently specified active ionic flux in the above theory, for example $J^a_{HCO_3^-}$ in the preceding section, is employed for simplicity [24]. The consideration of active ionic flux, and its coupling with the solvent and solutes can be elaborated by taking into account the underlying metabolic reactions [9]. This development would require identification of the molecular mechanisms, but could potentially enhance the understanding of active mechanism involvement in membrane transport.

Supporting Information

S1 Appendix. Derivations of the KK equations considering active transport and determinations of fluid pressure and solute concentrations at steady state.
(PDF)

Author Contributions

Conceived and designed the experiments: XC PMP. Performed the experiments: XC. Analyzed the data: XC PMP. Contributed reagents/materials/analysis tools: XC. Wrote the paper: XC PMP.

References

1. Titze J (2013) Interstitial fluid homeostasis and pressure: news from the black box. Kidney Int 84: 869–871. doi: 10.1038/ki.2013.287 PMID: 24172732

2. Jiang H, Sun SX (2013) Cellular pressure and volume regulation and implications for cell mechanics. Biophys J 105(3): 609–619. doi: 10.1016/j.bpj.2013.06.021 PMID: 23931309

3. Hoffmann EK, Lambert IH, Pedersen SF (2009) Physiology of cell volume regulation in vertebrates. Physio Rev 89(1): 193–277. doi: 10.1152/physrev.00037.2007

4. Stewart MP, Helenius J, Toyoda Y, Ramanathan SP, Muller DJ, Hyman AA (2011) Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. Nature 469: 226–230. doi: 10.1038/nature09642 PMID: 21196934

5. Charras GT, Yarrow JC, Horton MA, Mahadevan L, Mitchison TJ (2005) Non-equilibration of hydrostatic pressure in blebbing cells. Nature 435: 365–369. doi: 10.1038/nature03550 PMID: 15902261

6. Fraser JA, Huang CLH (2004) A quantitative analysis of cell volume and resting potential determination and regulation in excitable cells. J Physiol 559: 459–478. doi: 10.1113/jphysiol.2004.065706 PMID: 15243134

7. Jakobsson E (1980) Interactions of cell volume, membrane potential, and membrane transport parameters. Am J Physiol 238(5): 196–206.

8. Hodson SA (1971) Why the cornea swells. J Theor Biol 33(3): 419–427. doi: 10.1016/0022-5193(71)90090-7 PMID: 4262718

9. Schultz SG (1980) IUPAB Biophysics series. Basic principles of membrane transport. Cambridge University Press, Cambridge.

10. Friedman MH (2008) Principles and models of biological transport, second edition. Springer, New York.

11. Demirel Y, Sandler SI (2002) Thermodynamics and bioenergetics. Biophys Chem 97(2): 87–111. doi: 10.1016/S0301-4622(02)00069-8 PMID: 12050002

12. Kedem O, Katchalsky A (1958) Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. Biochim Biophys Acta 27(2): 229–246. doi: 10.1016/0006-3002(58)90330-5 PMID: 13522722

13. Kedem O, Katchalsky A (1963) Permeability of composite membranes Part 1. electric current, volume flow and flow of solute through membranes. Trans Faraday Soc 59: 1918–1930. doi: 10.1039/tf9635901918

14. Hoshiko T, Lindley BD (1967) Phenomenological description of active transport of salt and water. J Gen Physiol 50(3): 729–758. doi: 10.1085/jgp.50.3.729 PMID: 11526855

15. Kargol A (2000) Modified Kedem-Katchalsky equations and their applications. J Membr Biol 174(1): 43–53. doi: 10.1016/S0376-7388(00)00367-7

16. Li LY (2004) Transport of multicomponent ionic solutions in membrane systems. Philos Mag Lett 84(9): 593–599. doi: 10.1080/09500830512331325767

17. Overbeek JT (1956) The Donnan equilibrium. Prog Biophys Biophys Chem 6: 57–84. PMID: 13420188

18. Hodson S, Earlam R (1993) The incorporation of gel pressure into the irreversible thermodynamic euqation of fluid flow in order to explain biological tissue swelling. J Theor Biol 163(2): 173–180. doi: 10.1006/jtbi.1993.1115 PMID: 8246502

19. Urban JPG, Maroudas A, Bayliss MT, Dillon J (1979) Swelling pressures of proteoglycans at the concentrations found in cartilaginous tissues. Biorheology 16(6): 447–464. PMID: 534768

20. Niles WD, Cohen FS, Finkelstein A (1989) Hydrostatic pressures developed by osmotically swelling vesicles bound to planar membranes. J Gen Physiol 93(2): 211–244. doi: 10.1085/jgp.93.2.211 PMID: 2467961

21. Cheng X, Petsche SJ, Pinsky PM (2015) A structural model for the in vivo human cornea including collagen-swelling interaction. J R Soc Interface 12: 20150241. doi: 10.1098/rsif.2015.0241 PMID: 26156299

22. Wieth JO, Andersen OS, Brahm J, Bjerrum PJ, Borders CL Jr (1982) Chloride-bicarbonate exchange in red blood cells: physiology of transport and chemical modification of binding sites. Philos Trans R Soc Lond B Biol Sci 299(1097): 383–399. doi: 10.1098/rstb.1982.0139 PMID: 6130537

23. Blumenthal R, Caplan SR, Kedem O (1967) The coupling of an enzymatic reaction to transmembrane flow of electric current in a synthetic "active transport" system. Biophys J 7(6): 735–757. doi: 10.1016/S0006-3495(67)86620-7 PMID: 19210996

24. Klyce SD Russell SR (1979) Numerical solution of coupled transport equations applied to corneal hydration dynamics. J Physiol 292: 107–34. doi: 10.1113/jphysiol.1979.sp012841

25. Leung BK, Bonanno JA, Radke CJ (2011) Oxygen-deficient metabolism and corneal edema following epithelial hypoxia in the rabbit. Prog Retin Eye Res 30(6): 471–492. doi: 10.1016/j.preteyeres.2011.07.001 PMID: 21820076

26. Prigogine I (1967) Introduction to thermodynamics of irreversible process, third edition. Interscience publisher, New York.

27. Cheng X, Pinsky PM (2013) Mechanisms of self-organization for the collagen fibril lattice in the human cornea. J R Soc Interface 10: 20130512. doi: 10.1098/rsif.2013.0512 PMID: 23904589

28. Chhabra M, Prausniz JM, Radke CJ (2009) Modeling Corneal Metabolism and Oxygen Transport During Contact Lens Wear. Optom Vis Sci 86(5): 454–466. PMID: 19357551

29. Pinsky PM (2014) Three-dimensional modeling of metabolic species transport in the cornea with a hydrogel intrastromal inlay. Invest Ophthalmol Vis Sci 55(5): 3093–3106. doi: 10.1167/iovs.13-13844 PMID: 24833750

30. Elliott GF, Hodson SA (1998) Cornea, and the swelling of polyelectrolyte gels of biological interest. Rep Prog Phys 61: 1325–1365. doi: 10.1088/0034-4885/61/10/001

31. Bonanno JA (2012) Molecular mechanisms underlying the corneal endothelial pump. Exp Eye Res 95(1): 2–7. PMID: 21693119

32. Zeuthen T (1995) Molecular mechanisms for passive and active transport of water. Int Rev Cytol 160: 99–161. doi: 10.1016/S0074-7696(08)61554-5 PMID: 7558688

33. Kedem O, Katchalsky A (1961) A physical interpretation of the phenomenological coefficientsf of membrane permeability. J Gen Physiol 45(1): 143–179. doi: 10.1085/jgp.45.1.143 PMID: 13752127