Interaction of the Tear Film with the Ocular Surface

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IMA Workshop on Electrohydrodynamics and Electrodiffusion in Material Sciences and Biology
March 14, 2018

Supported by the NSF and the NIH
Why Study Human Tear Film?

Normal tear film dynamics: Many aspects to understand!

Dry eye syndrome: A common abnormality associated with the disruption of the tear film. It is caused by an insufficient or malfunctioning tear film.

Prevalence: It is estimated that 10% - 15% of Americans over the age of 65 have one or more symptoms of dry eye syndrome*.

Symptoms: Burning/stinging; Blurred vision
Irritation/redness; Dry sensation
Foreign body or “gritty” sensation; Tearing

Impact: Tasks of daily life such as reading and driving are negatively impacted by dry eye syndrome**.

**Miljanovic et al. 2007.
What is the Tear Film?

**Lipid layer** floating fatty/oil slick at interface with air
(≈ 50 – 100nm)

**Aqueous** mostly water between lipid and ocular surface
(≈ 3 – 5 μm)

**Ocular surface** Transmembrane mucins+microplicae (≤ 0.5 μm)

Gipson 04 via Nichols 85 (guinea pig)
Govindarajan & Gipson 10
Overview of Dynamics

Tear film supply and drainage

Lacrimal gland: The lacrimal gland supplies new tear fluid during a blink cycle.

Meibomian gland: The meibomian gland supplies lipids from lid edges.

Punctal drainage: Removes excess fluid starting at the halfway open position of the lids.

Typical blink cycle

Upstroke/Formation: Opening of lids, 0.1758s.

Interblink/Relaxation: Lids remain open, 5s (wide variation).

Downstroke: Closing of lids, 0.0821s.
Tear Film During a Blink

- Imaging the lipid layer during a blink: stroboscopic light source eliminates blur
- More colorful is thicker
- Moving lid plows the lipid layer into thicker state
- Not uncommonly, very similar structure reappears

Tear Film During a Blink

- Imaging the lipid layer during a blink: more colorful is thicker
- Moving lid plows the lipid layer into thicker state
- Rapid TBU visible (Lan Zhong et al MMB 17)
- Source: RJB et. al (2015), PRER
Tear Breakup (TBU), Begley et al (Indiana)

- Sodium fluorescein solution instilled as drop; quenching regime (Nichols et al 12)
- Glows green under blue light
- High concentration quenches: Darker if thinned by evaporation
- Significant regions of breakup as time increases (movie)

Tear Breakup (TBU), King-Smith et al (OSU)

- Interferometry aimed at lipid layer reveals more
- Light and dark contours show relative change in LL thickness
- Roughness appears in TBU regions
- Significant regions of breakup as time increases (movie)
Tear Film Dynamics: Simultaneous imaging

**Lipid+aqueous:** FL+Lipid IF images by King-Smith et al (IOVS 13)

- Top: 15s post-blink; bottom: 20 pb
- Lipid holes preceded dim FL areas.
- Elevated evaporation through holes
- High osmolarity in TBU estimated with math models (Peng et al ’14, RJB et al ’15, ’17)
Part I: Blinking and Rippling
Tear Film During a Blink

- Imaging the lipid layer during a blink: more colorful is thicker.
- Moving lid causes rough surface, echoing the cornea.
- Roughness of TF due to flow.
Modeling of a blink

- Ends of film move, thin layer under moving ends
- Evaporation doesn’t matter in short time of blink
- Tear/air interface is "uniformly stretching": limit of strong surfactant
  Jones et al, 05
  RJB and King-Smith, 07
- A simplistic "rough" cornea is assumed: similar to observations
  Gipson 2004
  King-Smith and Nichols 2014
Model for blinking domain

On \(-X(t) < x < X(t)\),

\[
\frac{\partial h}{\partial t} + \frac{1 - x}{1 - X(t)^2} \frac{h}{dt} dX - \frac{1}{12} \frac{\partial}{\partial x} \left( h^3 \frac{\partial p}{\partial x} \right) = 0,
\]

\[p = -\frac{\partial^2 h}{\partial x^2} - \frac{\partial^2 z_c}{\partial x^2}.
\]

- First eqn: PDE for TF thickness \(h\)
- Motion of whole surface induced via \(dX/dt\) term: uniform stretching
- Second eqn: definition of pressure
- Pressure is leading order part of curvature of TF surface
Model for blinking domain

At the ends,

\[ h(\pm X, t) = h_0 - z_c(\pm X) \]

\[ -\frac{h_0^3}{12} \frac{\partial p(X, t)}{\partial x} = \pm \left\{ -[h_0 - z_c(\pm Z)] + [h_e - z_c(\pm X)]/2 \right\} \frac{dX}{dt}. \]

- First eqn: Pin the TF thickness at constant value
- Second eqn, rt side: flux into or out of TF via motion of end
- Second eqn, left side: response of film at end
Nonlinear model results

- Ends of film move and drag fluid
- Moving film echoes corneal surface
- Like ripples on a shallow stream
Nonlinear model results

- Downstroke and upstroke shift peaks relative to corneal surface
- Seen in expt!
Nonlinear model results

- A set of dots did not move with lids or tear film
- Bright and dark sides flipped with direction of lids
- Consistent with phase shift
- Seen in theory!
Part II: TBU and Rippling

(Amy Janett et al, in prep, 2017)
Roughness in TBU

- Left: TFLL interferometry
- Below: Retroillumination (Begley lab, IU)

Braun et al, PRER 2015

How?
In interblink period now
Evaporation drives thinning here
Capillarity pushes flow into thin region but not fast enough if TBU
Corneal surface assumed to be wettable, with associated equilibrium thickness of glycocalyx
Scalings and nondimensionalization

Scalings based on tear break up (TBU):

- $v_0$ is measured peak thinning rate
- $d$ is characteristic thickness
- $d/v_0$ is time scale: TBUT for flat film
- surface tension $\gamma$, viscosity $\mu$
- balance surface tension and viscosity: $S = \gamma \epsilon^4 / (\mu v_0) = 1$

so that $\ell = \left( \frac{\gamma}{\mu v_0} \right)^{1/4} d$

For $v_0 = 20 \mu m/min$, $d = 3.5 \mu m$, $\ell \approx 0.35 mm \rightarrow \epsilon = d/\ell = 0.01 \rightarrow$

Lubrication theory
Model for TBU, rough cornea

For the TF thickness $h$:

$$
\frac{\partial h}{\partial t} - \frac{1}{12} \frac{\partial}{\partial x} \left( h^3 \frac{\partial p}{\partial x} \right) = -J,
$$

$$
p = -\frac{\partial^2 h}{\partial x^2} - \frac{\partial^2 z_c}{\partial x^2} - \Pi(h).
$$

For the Gaussian evaporation function $J(x)$, and wetting forces given by

$$
J(x) = \frac{v_1}{v_0} + \left( 1 - \frac{v_1}{v_0} \right) \cdot \exp \left( \frac{- (x - \frac{x_L}{2})^2}{2x_w^2} \right).
$$

Ocular surface function $z_c(x)$:

$$
z_c(x) = z_a \left[ 1 + \sin(2\pi kx) \right],
$$

Wetting forces $\Pi(h)$:

$$
\Pi(h) = Ah^{-3}.
$$

Flat IC’s:

$$
h(x, 0) = 1 - z_c(x) - z_a, \quad p(x, 0) = \Pi(h(x, 0)).
$$
Model for TBU, rough cornea

Typical parameter values are:

- $v_1/v_0 = 1/20$, slow evaporation around peak value (King-Smith et al 2005)
- $x_L = 8$, wide domain
- $x_w = 1.2$, evaporation width about natural scale
- $z_a = 1/14$, amplitude about same as glycocalyx, surface roughness
- $k = 10$, based on average corneal cell size of 36 $\mu m$
- $A = 9.9 \times 10^{-3}$, stops thinning at 0.25 $\mu m$ (glycocalyx size).

Numerical solution method similar except Fourier spectral in space
Film thinning fastest at peak evaporation
Surface roughness visible at TBU
Approximate $h_a$ computed at TBU

Approximate relative amplitude $r = h_a/z_a$ decreases with increasing $k$
Summary for rippling

- Lipid imaging system caught rippling during blink
- Can explain observations with flow over rough substrate
  - Shifting of image, scales
  - Nonlinear (shown) and linear theory (not shown)
- Simplified TBU model sees rippling quite close to TBU: good for experimenters!
- Generalizations: better model for glycocalyx (first effort started with Mastroberardino, Siddique, Anderson)
Part III: Osmolarity increase
(RJB, Nick Gewecke, et al, Prog Ret Eye Rsch, 2015;
Peng et al, Adv Coll Interface Sci 2014;
RJB et al Math Med Biol 2017;
Longfei Li et al, Math Med Biol 2016)
Observations

Overall dynamics

- King-Smith imaging (09)
- Sodium fluorescein solution instilled as drop; quenching regime (Nichols et al 12)
- Glows green under blue light; lighter is less concentrated (movie)
Osmolarity and Dry Eye Syndrome (DES)


- Osmolarity: concentration of ions (primarily salts and proteins) in aqueous.
- Osmolarity thought to be important in DES (DEWS Report, 2007)
- Tear film instability: evaporation, breakup (TBU)
- Elevated osmolarity: pain, inflammation, damage
- Water permeability of cornea less than conjunctiva
- Measurements only in temporal canthus; elsewhere?
- Estimate osmolarity in TBU from imaging or models?

Diagnose dry eye with TearLab
Model Formulation

PDEs for thickness $h$ and osmolarity $c$:

$$\partial_t h + \nabla \cdot Q = -EJ + P_c(c - 1)$$

$$J = \frac{1 - \delta \left( S\Delta h + Ah^{-3} \right) - \frac{Bi(1-T_\infty)h}{1+Bi h}}{K + \frac{h}{1+Bi h}}$$

$$Q = \frac{h^3}{12} \nabla \left( S\Delta h + Ah^{-3} - Gy \right)$$

$$h\partial_t c + \nabla c \cdot Q = \frac{1}{Pe_c} \nabla \cdot (h\nabla c) + EJc - P_c(c - 1)c$$

ICs and BCs:

- $h(x, 0) = \text{"flat-bottom bowl"}$
- $c(x, 0) = 1$
- $c|_{\partial\Omega} = 1$

Solute Parameters:

- $Pe_c \approx 9.6 \times 10^3$ is the Péclet number
- $P_c \approx 1.305 \times 10^{-2}$ is the water permeability of cornea

$c$ equation based on Jensen and Grotberg, 1993.
Steady BC with $E = A = 0$, Maki et al, 2010
Unsteady BC with all but $c$, Li et al, 2013
Permeability Distribution

We consider different water permeability for cornea (12.0 μm/s, 0.013 nondimensionally) and conjunctiva (55.4 μm/s, 0.06 nondimensionally).
During a blink, tear film (TF) and epithelium experience changes in thickness and osmolarity. The overall osmolarity is critical for maintaining the balance of the tear film. Stratified epithelium plays a role in these changes. Overall:

- Thickness left, osmolarity right.
- A black line forms from capillarity as a dark blue band.
- Tear film is thinner in the interior due to evaporation; it's worst on the cornea.
- Lower water permeability of the cornea leads to it being thinner.
- The highest osmolarity is over the cornea, with a max(c) approximately 1.5 or 450 mOsm, which is the threshold of feeling (Liu et al, 2009).
- Faster evaporation: max(c) approximately 6 or 1800 mOsm is possible with a thinning rate of 20 µm/min. This is similar to the Tear Breakup Time (TBU) (Peng et al, 2014; Braun et al, 2015).
- Estimates of 800-900 mOsm in the TBU (in vivo, Liu et al, IOVS '09).
Part IV: Osmolarity and the epithelium
(Jen Bruhns et al, in prep; Spencer Walker et al, in prep)
**What are Corneal Epithelia?**

**Corneal Epithelium:** The outermost layer of the cornea, which is composed of a single layer of **basal cells**, and multiple layers of stratified squamous epithelial cells.

*Figure:* Hogan, Alvarado and Weddell, 1971.
What are Corneal Epithelia?

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*Figure:* Hogan 1971.
How do tear film models deal with these cells?

**No Flux:** Some tear film models assume that there is no flux of ions or water at the corneal surface (Zubkov *et al.*, JFM 2012).

**Semipermeable Membrane:** Other models treat the corneal surface as a semipermeable membrane, where as the osmolarity of the tear film becomes large an osmotic flux of water crosses the cornea (e.g., Li *et al.* MMB 2016).
Modeling the Cornea as a Stack of Cells

**Water Transport Models:** Levin *et al.* (2004) modeled water transport in a stack of these epithelial cells.

**Strengths:** Keeps track of water in the entire stack.

**Weaknesses:** Ignores ion (salt) transport.

**Figure:** Levin *et al.* 2004. (mice)
Ion Transport Models: Levin et al. 2006 modeled ion transport throughout the epithelium.

The multilayered epithelium was modeled as a single layer.

Strengths: This model is able to keep track of ions throughout the compartments.

Weaknesses: Simplified water transport with osmolarity throughout the system (320 mOsM).

The stack of epithelium was modeled as single layer.

Figure: Levin et al. 2006. (mice)
The Model

Model Assumptions:
- The basolateral solutions has fixed composition.
- The evaporation rate is constant.
- There is no water transport through the paracellular space (tight junctions).
Mass Conservation

**Water Transport:** Governed by osmosis and evaporation.

\[ J_i^W = \nu_i \rho_i^w (\psi_{\text{out}} - \psi_{\text{in}}). \]

\[ J_e^W = 20 \times 10^{-6} \text{cm/s} \]
Mass Conservation (continued)


\[ J_i^x = p_i^x z_x \frac{V_i F}{RT} \frac{C_{in}^x - C_{out}^x \exp\left(-z_x \frac{V_i F}{RT}\right)}{1 - \exp\left(-z_x \frac{V_i F}{RT}\right)} \]

\[ 1 \]

\[ ^1 \text{Hille 1992} \]
Active Transport: Pumps 3 sodium ions out of the cell and 2 potassium ions into the cell.

$$J_{act} = \rho_{act} \left[ \frac{C_{in}^{Na}}{C_{in}^{Na} + K_{act}^{Na}} \right]^3 \left[ \frac{C_{b}^{K}}{C_{b}^{K} + K_{act}^{K}} \right]^2 \cdot 2$$

\(^2\text{Levin et al. 2006}\)
Mass Conservation (continued)

**Co-Transport:** Pumps 2 chlorine ions, 1 potassium ion, and 1 sodium ion per cycle.

\[
J_{co} = p_{co} \frac{C_{in}^{Na} C_{in}^{K} (C_{in}^{Cl})^2 - C_{b}^{Na} C_{b}^{K} (C_{b}^{Cl})^2}{\left[1 + \frac{C_{b}^{Na}}{k_{Na}}\right] \left[1 + \frac{C_{b}^{K}}{k_{K}}\right] \left[1 + \frac{C_{b}^{Cl}}{k_{Cl}}\right]^2} \cdot 3
\]

\[3\] Levin et al. 2006
Model System

From the given mass fluxes a system can be set up via mass conservation and Kirchoff’s laws (solved using ODE15s in MATLAB).

**Concentration (differential):**

\[
\frac{dC_i^x}{dt} = \left[ a_i^x J_i^{x,Net} - C_i^x \right] \frac{1}{h_i}.
\]

**Height (differential):** Ions do not contribute to the volume.

\[
\frac{dh_{cell}}{dt} = -J_A^W - J_B^W.
\]

\[
\frac{dh_{tf}}{dt} = J_A^W - J_e^W.
\]

**Kirchhoff’s Laws (algebraic):** Prevents charge buildup.

\[ I_A + I_P = 0 \quad \text{and} \quad I_B + I_P = 0. \]

\[ ^4 V_A, V_B, \text{ and } V_P \text{ are the algebraic variables.} \]
**Results**

**Ions:** In this model ion concentrations remain in similar ranges to the ion transport model by Levin *et al.* 2006.
- Even with the fixed osmolarity assumption removed, the various osmolarities throughout remain at high (due to evaporation), but physiological levels.

**Figure:** Osmolarity for short time (left), and long time (right).
Results (continued)

**Water:** It is clear that there are two time scales for water transport.

- The tear film equilibrates more slowly than the cell for short time, but continues changing long after the cell reaches an equilibrium.

**Figure:** Height for short time (left), and long time (right).
Results (continued)

**Water:** It is clear that there are two time scales for water transport.

- The tear film equilibrates more slowly than the cell for short time, but continues changing long after the cell reaches an equilibrium.

**Figure:** Height for short time (left), and long time (right).
Dealing With a Stack of Epithelium: Prior models deal with ion transport in a single epithelium. We hope to model an entire stack.

Known:
- Potential difference across individual layers (rabbit).
- Epithelial cells are polar.
- The dynamics of the stack as one layer.

Unknown:
- Transporters on each individual epithelium?
- Should the system even reach an equilibrium?

Figure: Klyce 1972 (rabbit)
Conclusion

We have some partial knowledge of the distribution of some water channels (aquaporins).

**Aquaporins:**
- More AQP1 in endothelium and stroma.
- More AQP3 at back of epithelium.
- More AQP5 and CFTR at apical side.

**Figure:** Levin *et al.* 2006.
The Model

Assumptions:

- since the transporters are largely unknown it is simplest to first model two identical cells on top of one another.
- The paracellular space has a fixed height \((h_1(t = 0)/1000)\).
Model System

This system is larger but is nearly identical the case for one cell. New state equations must be added to deal with the paracellular solution.

**Concentration (differential):**

\[
\frac{dC^x_i}{dt} = \left[ a_i^x J^x_{i,Net} - C^x_i \right] \frac{1}{h_i}.
\]

**Height (differential):**

\[
\frac{dh_a}{dt} = J^W_{A,1} - J^W_e.
\]

\[
\frac{dh_1}{dt} = -J^W_{A,1} - J^W_{B,1}.
\]

\[
\frac{dh_2}{dt} = -J^W_{A,2} - J^W_{B,2}.
\]

\[
J^W_{B,1} = -J^W_{A,2}.
\]

**Kirchhoff’s Laws (algebraic):**

\[
l_{A,1} + l_{P,1} = 0.
\]

\[
l_{B,1} + l_{P,1} = 0.
\]

\[
l_{A,2} + l_{P,2} = 0.
\]

\[
l_{B,2} + l_{P,2} = 0.
\]
Results

**Steady State:** When the evaporation rate is set to zero the entire system should be at an equilibrium.

- Ions are pumped into the paracellular space from active transport in the top cell which drives up the osmolarity and causes the system to drift away from physiological conditions at very large time.

**Potentials:** In mice the potential difference across the cornea should be -23 mV. In this model with two stacked the potential difference is about -49 mV.
Three cell layers

We attempted to adjust the parameters for a stack of cells with three layers using fminsearch. We found:

- Trying to optimize for keeping equilibria from one layer produced paracellular space much too mobile
- Using fminsearch as a heuristic to keep roughly equilibrium values gave reasonable values
- Lower values for ion transport at interior apical sides
- More permeability in basolateral paracellular spaces
- Similar apical and basolateral end values
- Stays very close to equilibrium if zero evaporation
- Reasonable dynamics with evaporation: voltage drop -85mV for 1 minute at 12µm/min thinning
- Need to refine with 4 cell layers for rabbit or mouse.
Conclusion for cell models

Understanding how the tear film interacts with ocular epithelium will be useful in gaining a better understanding of dry eye syndrome.

**Future Work Hopes To:**
- Find more info re distribution of channels in each cell later.
- Better channel/membrane models?
- Model the entire epithelium.

**Figure:** Levin *et al.* 2006.
Summary

- Brief survey of tear film imaging in blinking and interblink
- During blinks, pattern consistent with theory of flow over rough surface
- In TBU, theory confirms experiment assumption that rough pattern is TBU
- Osmolarity in tear film with evaporation: elevated, especially in TBU
- Effect of evaporating tear film on corneal epithelium: ion and water transport
- Increasing tear film osmolarity brings water out of and Na\(^+\) through epithelium
- Not discussed today:
  - Rapid TF breakup dynamics (Lan Zhong, finishing 2018)
  - Two-layer TF breakup (Mike Stapf, finishing 2018)
  - FL imaging models (several papers)
  - Blinking eye, "lens" shape: with J Brosch, TA Driscoll
  - Blinking eye, overlapping grids: with K Maki (RPI), TAD, et al
- CAMM for more info: www.mathandmedicine.org
Thank you!

**UD:** Back, l to r: Rich, Spencer Walker, Kevin Aiton.
Front, l to r: Chris Cornwell, Jerome Troy, Amy Janett, Mike Stapf.
Not Pictured: Lan Zhong, Toby Driscoll.

**Ohio State Optometry:**
Ewen King-Smith

**Indiana Optometry:**
Carolyn Begley and Adam Winkeler

**RPI:** Kara Maki

**RPI:** Bill Henshaw, Jeff Banks, Longfei Li

**PSU York:** Javed Siddique
A question for you all

How to treat this layer of mucins and proteins that allow water to pass, but not at least some ions? (Argueso et al, J Biol Chem 2009)