The Ocular Surface

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Current Research Techniques

**In vitro**
- Primary and cell-lines cultures
- Cornea and conjunctiva

**In vivo**
- Mouse experimental dry eye model
- Microbial Keratitis

**Clinical Studies**
- Samples the ocular surface/tear film
- Changes in protein and mRNA expression
Common Analysis Techniques

- Real-time RT-PCR
- Western blotting, gelatin zymography, immunohistochemistry
- Luminex and ELISA assays
- In vivo imagining in the mouse: HRT, OCT, SLE
Tear Film

• What is the tear film?
• Why is it important?
• What are its characteristics?
• What are its components/structure?
• What are some methods for clinical examination?
What is the tear film?

- Fluid secreted to protect the ocular surface from stress:
  - Chemical
  - Physical
  - Microbial
- Lubrication
- Serves as the first refractive surface of the eye
- AKA precorneal/preocular tear film

Modified from Pflugfelder, Dry Eye and Ocular Surface Disorders, Elaine Kurie Illustrations
What is the tear film?

- Exceedingly complex
- Composition is very dynamic to maintain homeostasis
- Behaves as a single dynamic functional unit with different compartments to create a hydrated gel
Tear Film: Why is it important?

- Essential for maintaining:
  - health of the cornea and conjunctiva
  - optical quality of the cornea

- Disruption leads to:
  - compromised corneal and conjunctival physiology
  - reduced visual performance and...
Tear film Characteristics

- Basal production: 0.5-2μl/min
- Blinking rate: 10-20/mins
- Thickness varies 40nm-46μm
- Tear volume: 4-10μl
Tear Types:
1. **Basal**: constitutive levels and reduced in dry eye
2. **Reflex**: Response to stimulation
3. **Emotional**: sadness or joy
4. **Closed-eye**: during sleep

Lacrimal gland secretions are reduced during sleep, there is an increased serum-derived proteins from conjunctival blood vessels
Mechanisms for tear film removal

Don’t let them see you cry!

- nasal drainage!
- evaporation
- conjunctiva absorption
A two-step process of tear film deposition through a blink has been proposed:

1. Step 1: the upper lid pulls a layer of tears over the cornea
2. Step 2: the lipid layer drifts upward, which may drag up aqueous tears along with it.

Tear turnover rate: 16 ± 5%/min
Schirmer Test and PRTT

**Schirmer Test**
- Gold Standard
- No anesthetic
- Closed eye for 5min
- Normal: >15 mm
- Mild-mod dry eye: 5-10 mm
- Severe dry eye: <5 mm

**Phenol Red Thread Test**
- Not commonly used
- No anesthetic
- Open eye for 15sec
- Normal: >20 mm
- Dry eye: 0-10mm
Tear Film Structure

Old theory – Wolff’s 3 layer model

Tear Film Structure

The anterior lipid layer provides stability by interacting with the mucin-aqueous phase.

Secretory mucins mix with the aqueous layer.

Membrane-associated mucins on the microplicae of the epithelium form the glycocalyx.

From Gipson IK, Argueso
Tear Film Structure

Lacrimal gland
Krause
Accessory lacrimal glands
Wolfing

Bulbar conjunctiva
Palpebral conjunctiva
Meibomian gland
Gland of Moll
Gland of Zeis

Superficial lipid layer
Aqueous layer
Mucin layer

Lipid
Mucous/Aqueous
Glycocalyx
Epithelium

Membrane-spanning mucin
Cleaved membrane-spanning mucin
Gel-forming mucin
Lysozyme

Immunoglobulin A
Transferrin
Defensin
Trefoil factor
**Tear Film Components**

<table>
<thead>
<tr>
<th>Tear layer</th>
<th>Origin</th>
<th>Components</th>
<th>Physiological functions</th>
</tr>
</thead>
</table>
| Lipid      | Meibomian gland  
Accessory lacrimal glands | Wax, cholesterol, fatty acid ester | Lubrication, prevention of evaporation, stabilization |
| Aqueous    | Lacrimal gland  
Accessory lacrimal gland | Water  
Electrolyte: Na⁺, K⁺, Cl⁻, HCO₃⁻, Mg²⁺  
Proteins: albumin, lysozyme, lactoferrin, transferrin, ceruloplasmin, immunoglobulins (IgA, IgG, IgE, IgM)  
Cytokines, growth factors: EGF, TGF-α, TGF-β1, TGF-β2, bFGF, HGF, VEGF, substance P  
Other: glucose, vitamins | Lubrication, antimicrobial, bacteriostasis, oxygen supply, nutritional supply, mechanical clearance, regulation of cellular functions |
| Mucous     | Conjunctival goblet cells, conjunctival epithelial cells, corneal epithelial cells | Sulfomucin, Sialomucin Complexes (SMC), MUC1, MUC4, MUC5AC | Lowered surface tension, stabilization of aqueous layer |
Tear Film: Lipid Component

- Anterior layer of the tear film is composed of meibomian oil
- Reduces evaporation by approximately 90% to 95%
- Sources: meibomian glands, glands of Moll, glands of Zeis
Tear Film: Lipid Component

- **Meibomian Glands** are large sebaceous glands
- Without direct contact to hair follicles
- Posterior to tarsal plates of upper and lower eye lids
- Another name for Meibomian glands?
Tear Film: Lipid Component

Meibomian Glands
visible through conjunctiva
ducts on lid margin
Meibomian Gland - ANATOMY

• **Length**
  • follows the tarsus

• **Number**
  • more in upper lid (30-40)
  • less in lower lid (20-30)

• **Volume**
  • higher in upper lid (26µl vs. 13µl)

Tear Film: Lipid Component
Tear Film: Lipid Component

- Meibomian Gland is complex arrangement of...
  - Orifice
  - Excretory duct
  - Central duct
  - Ductules
  - Acini
Tear Film: Lipid Component

- Progenitor cells
  - Constantly dividing in basal layer
  - Migrate toward center of acinus
- Stem cell source unknown
Tear Film: Lipid Component

- Holocrine glands
- Acini filled by secretory cell (meibocytes)
  - Basal cells (b)
  - divide (d)
  - mature (m) & accumulate lipids
  - disintegrate (des) and release the oily product (meibum)
Tear Film Structure: Lipid Component

- **Delivery**
  - Muscular contraction during lid movement
    - Riolans muscle
    - Orbicularis

- **Secretion**
  - Generates secretory force by holocrine secretion
  - continuous process
Tear Film: Lipid Component

- Vascular, neuronal, and hormonal influences
- VIP nerves are in direct contact with meibomian gland acinar cells
- Androgen and estrogen receptors, regulatory role for the gender hormones
## Two Phases of the Lipid Component

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Function</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-polar</strong></td>
<td>Barrier to water and contaminants, determines melting temperature of lipid layer</td>
<td><strong>Cholesterol esters (CE)</strong></td>
</tr>
<tr>
<td>Air-lipid</td>
<td></td>
<td>Wax esters (WE)</td>
</tr>
<tr>
<td>92% of the lipids</td>
<td></td>
<td>(Most abundant)</td>
</tr>
<tr>
<td><strong>Polar</strong></td>
<td>Optimal spreading over aqueous and facilitates spreading of non-polar fraction</td>
<td><strong>Phosphotidylcholine</strong></td>
</tr>
<tr>
<td>Lipid-aqueous</td>
<td></td>
<td>Phosphotidylethanolamine</td>
</tr>
<tr>
<td>8% of the lipids</td>
<td></td>
<td>Glycoproteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free fatty acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol</td>
</tr>
</tbody>
</table>

![Diagram of air-lipid interface and two phases of lipid component](image)
Tear Film Structure: Lipid Component

• The color and brightness of the interference images are analyzed to yield lipid layer thickness

• The thickness of the lipid layer has been reported to be from 15-157 nm,
Tear film Characteristics

- Stabilizes the tear film and retards tear evaporation
- Fluorescein break up time (FBUT) enhance visibility of the tear film
- One of the most commonly used subjective diagnostic tests in clinical practice
Tear Break Up Time (TBUT)

- Fluorescein (FTBUT)
  - 1 drop preservative-free saline
  - Variable volumes delivered to ocular surface can disrupt the tear film
    - >10 sec normal
    - <10 sec = indicates dry eye
    - ≤ 5 sec = dry eye
• **Tear Break Up Time (TBUT)**

  - Non-Invasive (NITBUT)
    - *No fluorescein!!*
  - Possible Instruments
    - Oculus Keratograph 5
    - Medmont Corneal Topographer
  - >15 sec normal
  - <15 sec= indicates dry eye
Not all TBUT are the same.

NITBUT values from Medmont were 43% greater than Oculus Keratograph (Lin et al., 2013).

NITBUT is significantly longer than FTBUTs (Weizhong et al., 2014).
Tear Film Structure: Lipid Component

Fig. 2. Tear film lipid layer interferometry grading patterns. From Yokoi et al., Correlation of tear lipid layer interference patterns with the diagnosis and severity of dry eye. Am J Ophthalmol. 1996; 122: 818-24 [35]. A = Grade 1 (gray uniform), B = Grade 2 (gray non-uniform), C = Grade 3 (few colors non-uniform), D = Grade 4 (many colors non-uniform), E = Grade 5 (partly exposed corneal surface).
Tear Film Structure: Lipid Component

Grade 1  Grade 2  Grade 3  Grade 4  Grade 5
Meibomian glands are thought to be hyper-reflective from infrared light.

It has been hypothesized that dropout is due to atrophy or might actually be the more anterior anatomical position of the glands.
Meibomian Glands

Meibography described by Yokoi et al.,

Fiber Optic Transilluminator

Drop Out
Oculus Keratograph - Meibography
Oculus Keratograph - Meibography
Mouse Meibomian Glands

Dry Eye Mouse (OD and OS)

Control Mouse (OD and OS)
Mouse Meibomian Glands
Tear Film: Lipid Component Summary

• Outmost, superficial layer of the tear film

• Functions:
  1) Reduces evaporation
  2) Stabilize the tear film
  3) Barrier to pathogens

• Complex mixture
Want More?

- Meibomian Gland Dysfunction “Bible”
- March 2011
Tear Film: Aqueous Component

• Functions:
  – immune defense
  – Wound healing
  – Stability promoting
  – Enzymes
  – Metabolic
  – Structural/mechanical
  – Cell signaling
  – Cell adhesion
  – Transport
Tear Film: Aqueous Component

- Proteins, electrolytes, ions
- Sources:
  - Lacrimal gland (LG)
  - Accessory LGs
    - Glands of Krause
    - Glands of Wolfring
Tear Film: Aqueous Component
Tear Film: Aqueous Component

Lacrimal gland responds rapidly to reflexes to maintain homeostasis:
- mechanical
- chemical
- thermal
Tear Film: Aqueous Component

Stimuli results in...

- Nerves to release neurotransmitters
- Mostly Parasympathetic (Ach/VIP)
- Some Sympathetic (NEPI)
- Some Sensory (SP, CGRP)
- Hormonal influences

Touch ➔ Ophthalmic branch ➔ trigeminal nucleus ➔ BLINK! ➔ CN VII ➔ facial motor nucleus
Tear Film: Aqueous Component

**Electrolytes and ions**
- Responsible for tear osmolality
- Membrane permeability
- pH 7.5-7.8 (open)
- pH 6.9 (closed)
- Osmolality ~290-310mOsm/Kg

<table>
<thead>
<tr>
<th>ION</th>
<th>(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>120-160</td>
</tr>
<tr>
<td>Cl</td>
<td>118-135</td>
</tr>
<tr>
<td>HCO3</td>
<td>20-25</td>
</tr>
<tr>
<td>K</td>
<td>20-42</td>
</tr>
<tr>
<td>Mg</td>
<td>0.7-0.9</td>
</tr>
<tr>
<td>Ca</td>
<td>0.5-1.1</td>
</tr>
</tbody>
</table>
Tear osmolarity

Measure osmolarity of the inferior tear meniscus

Spikes in tear osmolarity may be the source of burning sensation in Dry Eye sufferers

How salty or concentrated are the tears?
Tear Osmolarity

Tear film changes
• Quickly
• Constantly drained/turned over

May not fully reflect changes that occur over the corneal surface during the interblink interval

Spikes in tear osmolarity may be the source of burning sensation in Dry Eye sufferers
Tear osmolarity

Should we take tear osmolarity “with a grain of salt”? 

![Graph showing tear osmolarity over time for Dry Eye and Normal conditions.]
Tear osmolarity

- Tear osmolarity levels may vary... but...
- Hyperosmolar stress can increase proinflammatory cytokines (i.e., IFN$\gamma$) that are associated with ocular surface staining
Proteins

• 1500 proteins!!

• Total protein 3-10 µg/µl

• Produced by the lacrimal gland

• Some derived through capillary leakage
### Table 4.1 Functional peptides of tears

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>Refs</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal growth factor (EGF)</td>
<td>51, 52, 53, 54, 55</td>
<td>Epithelial wound healing.</td>
</tr>
<tr>
<td>Transforming growth factor alpha (TGF-α)</td>
<td>56, 57</td>
<td>Tear concentration higher than saliva or serum</td>
</tr>
<tr>
<td>Transforming growth factor beta-1 (TGF β-1)</td>
<td>58, 59</td>
<td>Wound response</td>
</tr>
<tr>
<td>Transforming growth factor beta-2 (TGF β-2)</td>
<td>58, 60, 61</td>
<td>Wound response</td>
</tr>
<tr>
<td>Hepatocyte growth factor (HGF)</td>
<td>62, 63</td>
<td>Found in normal tears, increases after wounding</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (FGF-2)</td>
<td>64</td>
<td>Wound response</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>60, 65</td>
<td>Wound response, increases after wounding</td>
</tr>
<tr>
<td>Platelet derived growth factor-BB</td>
<td>65, 66</td>
<td>Did not change after PRK</td>
</tr>
<tr>
<td><strong>Neuropeptides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P</td>
<td>14, 67, 68</td>
<td>Wound healing, neurogenic inflammation</td>
</tr>
<tr>
<td>Calcitonin gene related peptide</td>
<td>60, 69</td>
<td>Wound healing, neurogenic inflammation</td>
</tr>
<tr>
<td><strong>Interleukins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>72</td>
<td>Increases in vernal conjunctivitis</td>
</tr>
<tr>
<td>IL-1α, IL-1β</td>
<td>73, 74</td>
<td>Elevation of IL-1 in dry eye patients</td>
</tr>
<tr>
<td>IL-2, IL-4, IL-6, IL-8, IL-10</td>
<td>75, 76, 77</td>
<td>Increases with contact lens wear, ocular allergy</td>
</tr>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA, IgE, IgG(1–4) and complement</td>
<td>20, 78</td>
<td>Ocular allergy</td>
</tr>
<tr>
<td><strong>Proteases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-1, MMP-3, MMP-9, TIMP-1, capthepsin, alpha2-macroglobulin</td>
<td>79, 80, 81, 82</td>
<td>Role in pterygium migration and vernal keratoconjunctivitis, protection of the ocular surface</td>
</tr>
<tr>
<td><strong>Antimicrobial Peptides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme, lactoferrin, α and β defensins, phospholipase A2</td>
<td>83, 84, 85, 86, 87, 88</td>
<td>Increases in infections, wound healing, may decrease in dry eye</td>
</tr>
</tbody>
</table>
Tear Film: Aqueous Component

Lyzozyme
- 20-40% of tear protein!!
- 1-2ug/ul
- Antimicrobial
- breaks the bond (red arrow) between sugars bonds that make up the backbone of the peptidoglycan chains in bacterial cell wall
Tear Film: Aqueous Component

Lysozyme
- Degrades cell wall
- Bacteria succumbs to osmotic gradient
Lactoferrin and transferrin
- Iron binding proteins
- Sequesters essential iron from microbes replication
Tear Film: Aqueous Component

Phospholipase A2
• Powerful enzyme
• Breaks down phosphotidylglycerol which is main lipid components of the inner bacterial membrane
• Gram + bacteria!
IgA

- Major antibody present in seromucous secretions
  - e.g. saliva, tears where is in form of a dimer called secretory IgA
- half-life: 6 days
Lipocalin

- AKA tear-specific prealbumin
- Binds lipids to help stabilize the tear film
- Sequesters harmful lipidphilic molecules to prevent epithelial interaction
Antimicrobial Peptides (AMP)

• Defensin subgroups:
  • α-defensins (HNP): neutrophils
  • β-defensins (hBD): epithelia

• Cathelicidin (LL-37): epithelia and neutrophils

• Typically have broad spectrum of antimicrobial activity

Are AMP coated contact lenses/cases in our future?
Tear Film: Aqueous Component

- Recognize endogenous and microbial ligands
- TLR activation stimulates antimicrobial peptide and cytokine production
Tear Film: Mucin Component

- Secreted mucins and epithelial glycocalyx

- General Functions
  - Protection (gel-forming barrier)
  - Lubrication

- Expression in many cancers and lung disease (cystic fibrosis)
Tear Film: Mucin Component

- Bulbar and palpebral conjunctival goblet cells
- Stratified squamous epithelial cells of the cornea and conjunctiva
- Mucus producing cells decrease with age, inflammatory conditions, and vitamin A deficiency, contact lens wear
Tear Film: Conjunctiva

- Non-keratinized squamous
- 2-10 layers
- microvilli/glycocalyx
- Stem cells
- Other cell types:
  - Goblet
  - Melanocytes
  - Langerhans
Tear Film: Mucin Component
O Goblet cell, Where art thou?

- 1.5 million are distributed over the conjunctival surface
- most over the nasal conjunctiva
- least in the superior temporal bulbar conjunctiva

Mucins - MUCs

- MUC1, 2, 3, 4, 5AC, 5B, 6-9, 11-13, 15-19

- Rich in serine/threonine (TR)
  - 50-80% mass is carbohydrate

- Secreted or Membrane bound

<table>
<thead>
<tr>
<th>MUCIN</th>
<th>FUNCTION</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1, -4, -16</td>
<td>Membrane-bound mucins</td>
<td>Corneal epithelial surface</td>
</tr>
<tr>
<td>MUC5AC</td>
<td>Gel-forming mucins</td>
<td>Dissolved within the aqueous</td>
</tr>
<tr>
<td>MUC7</td>
<td>Soluble mucins</td>
<td>Associated with outer lipid component of aqueous</td>
</tr>
</tbody>
</table>
Tear Film: Mucin Component

- sensory nerves induces goblet cell secretion by stimulating a local reflex arc
- parasympathetic neurotransmitters, have been shown to stimulate goblet secretion
- Gender hormones are also known to influence goblet cell density

Tear Film: Mucin Component

Secreted Mucins

1) Gel-forming mucins (Large)
   - MUC5AC, 5B, 6, & 19
     - Expressed by goblet cells
     - Responsible for fluid properties of mucus

2) Soluble mucins (Small)
   - MUC2, 7 and 9
   - MUC7 secreted by lacrimal gland acinar cells

TR = tandem repeats
D (MUC5AC) = cysteine domains
(MUC7) = histatin-like domains (Hsn) antimicrobial properties
Tear Film: Mucin Component

Membrane Associated Mucins (MAMs)

- MUCs 1, 3A, 3B, 4, 12, 13, 15-17, 20
- Extend from cell surface to form glycocalyx
  - ~ 200-500 nm long
- Characterized by ectodomains (extracellular) & cytoplasmic domains (associated with actin cytoskeleton)
MUC localization

- MUC1,4,16 on ocular surface epithelial cells
- Corneal Epithelium: Express MUC on superficial epi
- Conjunctiva Epithelium:
  - Express MUC on superficial and wing cells
  - Goblet cells produce MUC5AC
Mucins function:

- Fluid properties of tears (mostly MUC5AC)
- Lubrication
- Protection
- Wetting of Ocular Surface
- Reservoir for Ig and defense molecules
- Prevents bacterial attachment
Tear Film: Mucin Component

Transmembrane mucins attached to the microplicae of the epithelial cells extend into the tear film.

The apical cells have transmembrane mucins:
1. increase the adhesion tension for water
2. facilitating the spread of the tears
3. line of defense for the epithelial cells against infection and injury
Tear Film: Mucin Component
Tear Film: Mucin Component
Tear Film: Mucin Component

- Secreted mucins and epithelial glycocalyx
- General Functions
  - Protection
  - Lubrication
- Mostly parasympathetic control
Tear Film: Why is it important?

- Physical Protection
- Antimicrobial protection
- Lubrication
- Refraction
- Nutrition
Tear Film: Why is it important?

- Physical Protection
  - Superficial lipids repel foreign material
- Blinking removes particles in the tear film
Tear Film: Why is it important?

- Antimicrobial protection
  - IgA and IgF
  - Lysozyme
  - Lactoferrin and lipocalin
  - Antimicrobial peptides
  - Glycocalyx
  - Growth factors
Tear Film: Why is it important?

- Lubrication
  - Prevents desiccation that can cause discomfort, erosions and ulcerations

www.eyehealthweb.com
Tear Film: Why is it important?

- Refraction
  - Coats small corneal irregularities
- Smooth refractive surface
- Refractive index: 1.357
Nutrition
- Cornea is avascular
- Transportation of oxygen into the cells is a primary function of the tear film
- Other nutrients from aqueous
Tear Film Summary

- Fluid secreted to protect the ocular surface from stress
  - Chemical, microbial, physical, hypoxia
- Lipid layer = meibomian glands
- Aqueous = lacrimal gland
- Mucin = goblet cells
- Mostly parasympathetic control
- Hormonal influences
Tear Film

- What is the tear film?
- Why is it important?
- What are its characteristics?
- What are its components/structure?
- What are some methods for clinical examination?
Dry Eye
Dry Eye Definition

The first definition of dry eye, published in 1995 on the basis of consensus from the NEI/Industry working group on Clinical Trials in Dry Eye, was as follows:

“Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort.”
Dry Eye Definition

“Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.”

2007 DEWS Report
Dry Eye Definition

“Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.”

2017 DEWS II Report
Dry Eye Inflammation

Pathophysiology

- ↓ tear volume
- ↑ hyperosmolar stress
- ↑ cytokine and matrix metalloproteinase (MMP)
- ↑ ocular surface damage
Dry Eye Classification

**NEI / INDUSTRY WORKSHOP**
**1995**

**CLASSIFICATION OF DRY EYE**

**DRY EYE - KCS**

- Tear-deficient
  - Sjögren Syndrome
  - Non-Sjögren Tear Deficient
    - Lacrimal Disease
    - Lacrimal Obstruction
    - Reflex
  - 1°
  - 2°

- Evaporative
  - Oil Deficient
  - Lid Related
  - Contact Lens
  - Surface Change
  - 1°
  - 2°

**Key Terms**
- RA = rheumatoid arthritis
- SLE = systemic lupus erythematosus
- SyScI = systemic sclerosis
- 1° Bil = primary biliary cirrhosis
- Weg = Wegener’s granulomatosis
- Other = other autoimmune diseases
- N.P. keratitis = neuroparalytic keratitis
- PLD = primary lacrimal gland disease
- MGD = meibomian gland disease
- BLINK = blink abnormalities
- APERT = aperture abnormalities
- CONGR = lid surface incongruity
Dry Eye Classification

Aqueous-deficient
- Sjogren Syndrome Dry Eye
  - Primary
  - Secondary
- Non-Sjogren Dry Eye
  - Lacrimal Deficiency
  - Lacrimal Gland Duct Obstruction
  - Reflex Block
  - Systemic Drugs

Evaporative
- Intrinsic
  - Meibomian Oil Deficiency
  - Disorders of Lid Aperture
  - Low Blink Rate
  - Drug Action (Accutane)
- Extrinsic
  - Vitamin A Deficiency
  - Topical Drugs Preservatives
  - Contact Lens Wear
  - Ocular Surface Disease (eg, Allergy)
Dry Eye Inflammation

- Prevalence ranging from 7-33% (Gayton, 2009)

- 3X more likely to report a reduced ability to perform daily activities (WHS and PHS)

- A leading cause to seek eye care (Brewitt and Sistani, 2001)
Mucins are reduced in DES
Innate Molecule in Dry Eye

- ↓ Protective molecules
  - Lysozyme
  - Lactoferrin
  - Lipocalin
  - sIgA
  - Mucins
- ↑ Antimicrobial peptide, hBD2 and sPLA2
- Potential changes in TLR expression
Toll-Like Receptors (TLRs)

- Potential mechanism of dry eye inflammation
- TLR activation stimulates the production of antimicrobial peptides, cytokines and MMPs
- TLR1-10 expressed on the ocular surface
Toll-Like Receptors (TLRs)

- Potential mechanism of dry eye inflammation

- TLR activation stimulates the production of antimicrobial peptides, cytokines and MMPs

- TLR1-10 expressed on the ocular surface

- TLR expression is modulated in DED
Dry eye modulate TLRs?

1. Hyperosmolar Stress
2. Dry Eye Associated Cytokines
3. Desiccation: Cornea Organ Culture Model
4. Dry Eye Patients

- Desiccation
- Hyperosmolar Stress

**TLR expression and activation**

**Cytokines**

- MMP-9

**Epithelial Damage**

- Goblet cell loss
Are TLR modulated in EDE?

EDE model:

- C57BL/6 mice
- subcutaneous scopolamine injection (2.5 mg/ml) QID
- low humidity & air draft for 5 days

Experimental dry eye

Increase in TLR expression

More inflammation
TLR and Dry Eye Inflammation

Key Finding:
TLR2,3,4,5,9 expression is modulated on the cornea/conjunctiva of C57BL/6 mice with experimental dry eye

Redfern et al., IOVS, 2013
TLR and Dry Eye Inflammation

TLR4 inhibition decreases corneal staining and cytokine production in experimental dry eye.

Lee et al. IOVS 2012.
Functional Consequence?

EDE for 5D

TLR Agonist (OD)  Vehicle (OS)  EDE for 1D

Live In vivo Imaging
1. OCT Imaging
   • Fluorescein staining
   • Corneal thickness

Controls
1.) scratch (n=3)
2.) UT control (n=6)

Vivarium for 1D
TLR Increases EDE Corneal Damage
What is the mechanism involved in creating TLR induced corneal ulcers in dry eye?
Hyperosmolar stress causes the release of MMP-9 from neutrophils and epithelial cells.

MMP-9 disrupts ocular surface integrity.

Lanza et al., The Ocular Surface, Volume 14, Issue 2, 2016, 189–195
Tear Inflammation: MMP-9

- MMP-9 levels of more than 40 ng/ml
- Messmer et al. (2016) found a positive result correlates with:
  - Higher OSDI scores
  - TBUT ≤5 seconds
  - Conjunctival and corneal staining
  - Obstructed MG ducts
Do TLRs stimulate MMPs?

Methods:

HCEC were harvested from human cadaver corneas

\[ \text{TFR pathogenic agonists for 24hrs} \]

\[ \text{MMP protein detection and activity} \]

A. MMP Protein Detection

B. Gelatin Zymography
Additional Laboratory Aims

- **DRY EYE**
  - ↓ Tear Film
  - Hyperosmolar stress

- **PATHOLOGICAL**
  - ↑ Cytokines
  - ↑ MMP/OS Damage
  - ↑ infection ?

- **PROTECTIVE**
  - ↑ Antimicrobial Peptides
  - ↓ infection ?

- **Damage Associated Molecular Patterns**
  - or

- **Pathogen Associated Molecular Patterns**

- **TLR Activation**
TLR and Dry Eye Inflammation

- Hyperosmolar stress causes ocular surface damage
- Damage associated molecule patterns (DAMPs) are released
  - e.g. high-mobility group box-1 (HMGB1)
- HMGB1 is increased in Sjögren's syndrome (Dupire et al. 2012)
- Inhibition of DAMPs reduces disease severity

Karikó K et al, 2004
High Mobility Group Box-1 (HMGB1)

<table>
<thead>
<tr>
<th>Toll-like receptor</th>
<th>Exogenous ligands</th>
<th>Disease/injury</th>
<th>Potential ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1/2/6</td>
<td>Lipopeptides</td>
<td>Atherosclerosis, inflammatory</td>
<td>Fatty acids, Microbial antigens</td>
</tr>
<tr>
<td></td>
<td>Peptidoglycan</td>
<td>bowel disease</td>
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<tr>
<td></td>
<td>Lipoteichoic acid</td>
<td></td>
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<tr>
<td></td>
<td>Malp-2</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Zymosan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA</td>
<td>Type 1 diabetes, Rheumatoid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>arthritis</td>
<td></td>
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<tr>
<td>TLR4</td>
<td>LPS</td>
<td>Atherosclerosis, Multiple</td>
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<td></td>
<td></td>
<td>sclerosis, Asthma</td>
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</tr>
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<td></td>
<td></td>
<td>Rheumatoid arthritis</td>
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</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>Inflammatory bowel disease,</td>
<td></td>
</tr>
<tr>
<td>TLR7/8</td>
<td>ssRNA</td>
<td>Systemic lupus erythematositis,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type 1 diabetes</td>
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</tr>
<tr>
<td>TLR9</td>
<td>Unmethylated CpG DNA, DNA sugar</td>
<td>Inflammaratory bowel disease,</td>
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High Mobility Group Box-1 (HMGB1)

- Previously thought to only function as a nuclear factor to enhance transcription
- Under stress conditions, such as injury or infection, HMGB1 is released and promotes inflammation
- HMGB1 binds to TLR agonists (PAMPs) thereby potentiating the inflammatory responses
High Mobility Group Box-1 (HMGB1)
Blocking HMGB1 reduced PA keratitis severity

McClellan et al., *Journal of Immunology*, 2015
HMGB1 in Ocular Inflammation

Vernal Conjunctivitis (serum)

Dry Eye Syndrome (tears)

Zicari et al., Pediatric Allergy Immunology, 2014
HMBG1 and Experimental Dry Eye

A

Relative Fold Change

0 1 2 3 4

UT EDE

B

UT EDE

C

Pixel intensity

0 200000 400000 600000 800000

UT EDE
HMBG1 is Increased

A

UT

HOS (450 mOsM)

TNFα (10ng/ml)

B

HMBG1 (ng/ml)

0 5 10 15 20

UT 1h 6h

HMGB1 (ng/ml)

0 20 40 60 80 100 120 140 160 180 200 220 240

UT 400mOsM 450mOsM 500mOsM

6h 12h 24h

*** *** * *** ***

* *** ** ** ** **
HMBG1 Treatment

A

hTCEpi

Relative Fold Change

UT
4h
8h

IL-6
IL-8
TNFα

B

hTCEpi

pg/ml

IL-6
IL-8

C

Mφ-U937

Relative Fold Change

***
**

IL-6
IL-8
TNFα

D

Mφ-U937

pg/ml

IL-6
IL-8
TNFα

*
### Table 1

Toll-like receptors have been shown to be involved in inflammatory diseases and injury.

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<td>TLR9</td>
<td>Unmethylated CpG DNA DNA sugar backbone</td>
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</table>

**HSPs**  
- Chaperone proteins  
- Bind antigens and present them to the immune system
All together now

Hyperosmolar Stress

OS damage & instability

↑Cytokines
↑MMPs

Dry Eye

TLR activation

↑HMGB1

X
TLR deficiency and DES

- The activation of most TLRs are dependent on MyD88

- Will MyD88 knock out mice be more susceptible to dry eye inflammation?
Ocular Surface Damage

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>IL-1R -/-</th>
<th>MyD88 -/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td><img src="untreated_wild_type.png" alt="Image" /></td>
<td><img src="untreated_il-1r_-/-_.png" alt="Image" /></td>
<td><img src="untreated_myd88_-/-_.png" alt="Image" /></td>
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<tr>
<td>Desiccating Stress</td>
<td><img src="desiccating_wild_type.png" alt="Image" /></td>
<td><img src="desiccating_il-1r_-/-_.png" alt="Image" /></td>
<td><img src="desiccating_myd88_-/-_.png" alt="Image" /></td>
</tr>
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Ocular Surface Damage

Fluorescein Staining

Pixel Intensity

C57 | IL1R-/ | MyD88-/ |
---  | ---  | ---   |
Non-EDE | EDE  | Non-EDE | EDE  | Non-EDE | EDE  |

* | * | ns
Phenol Red Thread Test

Wicking distance (mm/15 sec)

<table>
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Ocular Surface Damage

Untreated

WT

IL-1R -/-

MyD88 -/-

EDE
Key Findings

- DAMPs are increased in response to hyperosmolar stress but do not stimulate cytokine or MMP production
- MyD88 is involved in DES ocular surface inflammation