

Preventing Corneal Ulcers Through Anti-Biofilm Networks on Contact Lenses

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6.3.2015

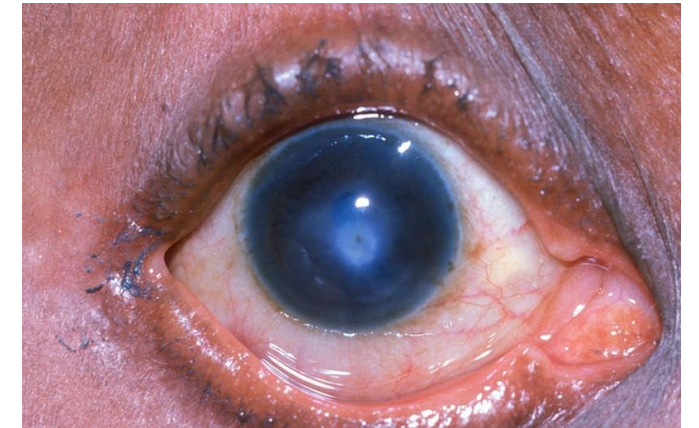
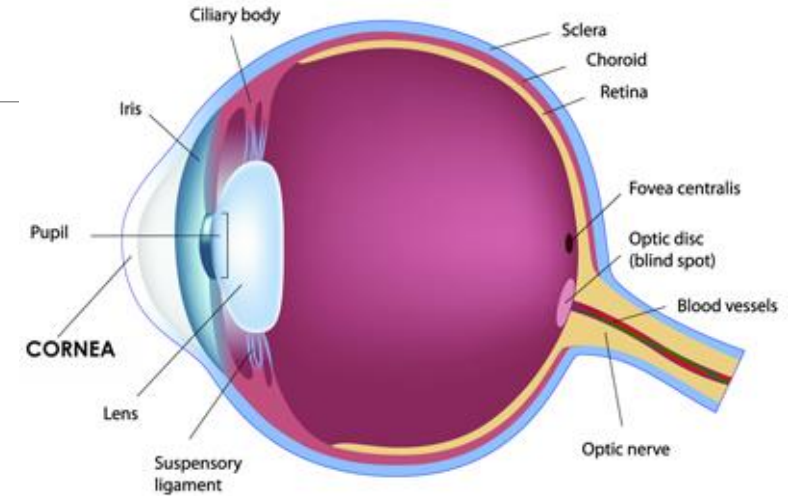
Outline

- Keratitis infections & corneal ulcers background information
- Current therapies & treatment
- Rationale & strategy for novel treatment idea
- Advantages & potential problems
- Development & manufacturing



Keratitis Infections and Corneal Ulcers

- Infection of the cornea
- Causes pain, reduced vision, light sensitivity and tearing or discharge from the eye
- If untreated causes corneal ulcers and even blindness
- Bacterial keratitis develops very quickly (can be caused by fungi, parasites, virus, or injury as well)
- Most common: *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*
- Estimated 7-25% of contact lens-users can be affected



Corneal ulcer (foggy white region) as a result of bacterial infection.

Treatment

- Prevent scarring of the cornea immediately
- If cause unknown, give antibiotic drops that work against many kinds of bacteria (ie. tobramycin (14 mg/mL) 1 drop every hour alternating with fortified cefazolin (50 mg/mL) 1 drop every hour)
- Severe ulcers may require corneal transplant
- Do not wear contact lenses and prevent by caring for contact lenses properly

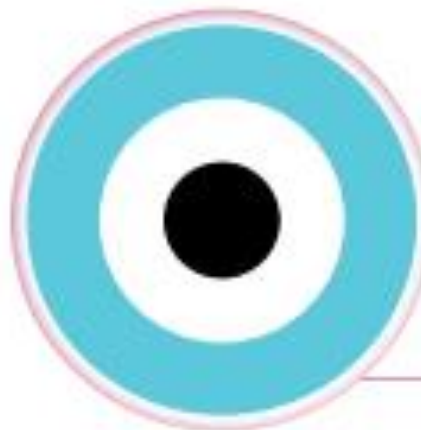


THE DANGERS OF CONTACT LENS MICROBIAL COLONISATION

For every 10,000 contact lens wearers:



>5 individuals will experience an eye infection



500 individuals will be affected by eye inflammation

Contact lens wear is the most common risk factor for corneal infection (keratitis) in developed countries:



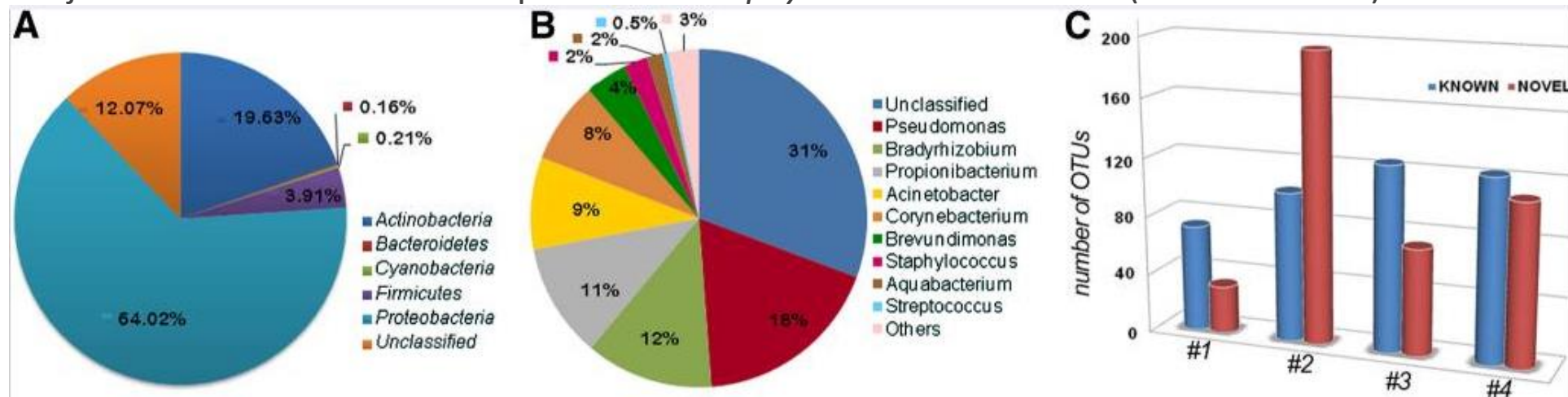
1 in 2,500 people who wear contact lenses and remove them at night will contract keratitis



1 in 500 people who wear contact lenses and sleep in them at night will contract keratitis

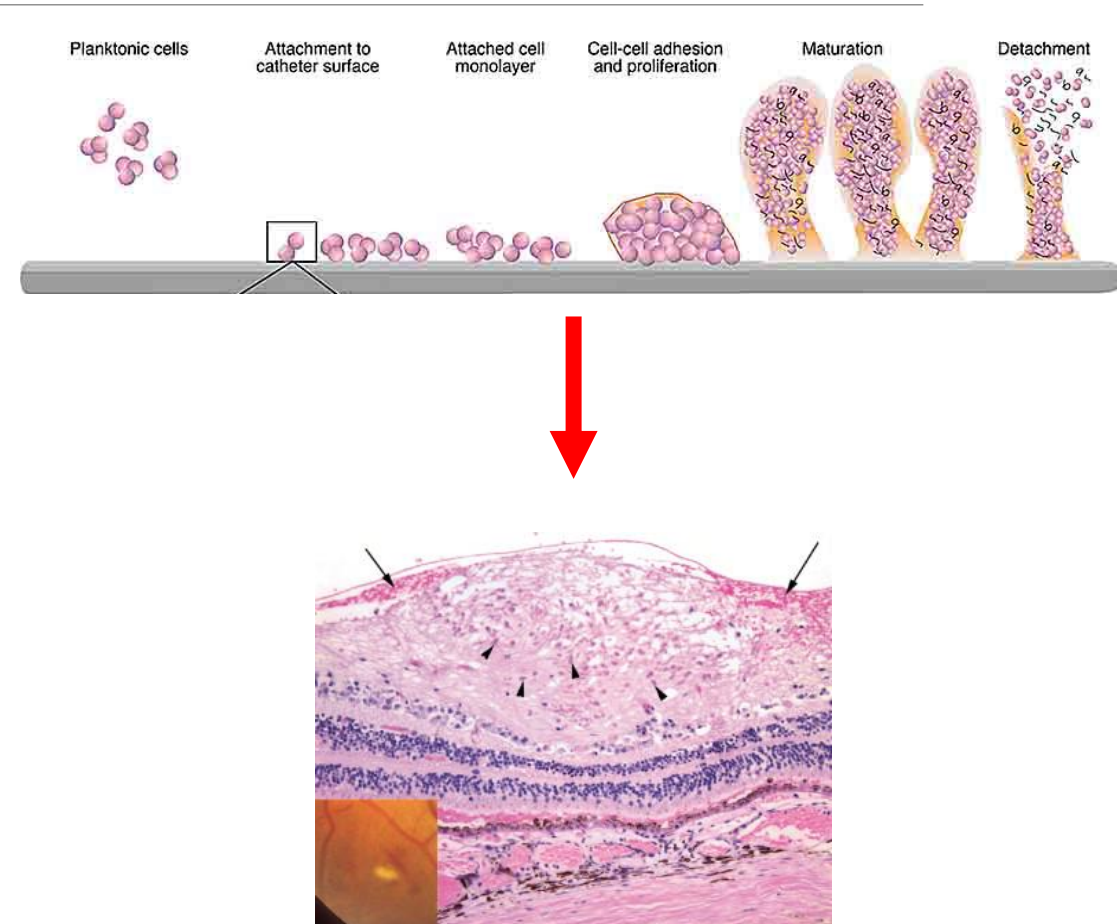
Ocular Microbiome Project

- 2009: Began by Dr. Shestopalov of the Bascom Palmer Eye Institute
- Prior belief: Not much of a microbial life, tears & blinking clear away foreign objects
- Found: All exposed mucosal epithelium are populated densely
 - Via 16s ribosomal RNA sequencing of: healthy corneas, contact lenses, and conjunctiva (inner surface of eyelids) & BRiSK analysis found during keratitis infections (and contact lens users) less bacterial diversity, mostly *Pseudomonas* strains (3 fold increase), and many of the bacterial genera that dominate the conjunctiva and cornea were depleted and *Staphylococcus* dominated (3 fold increase)



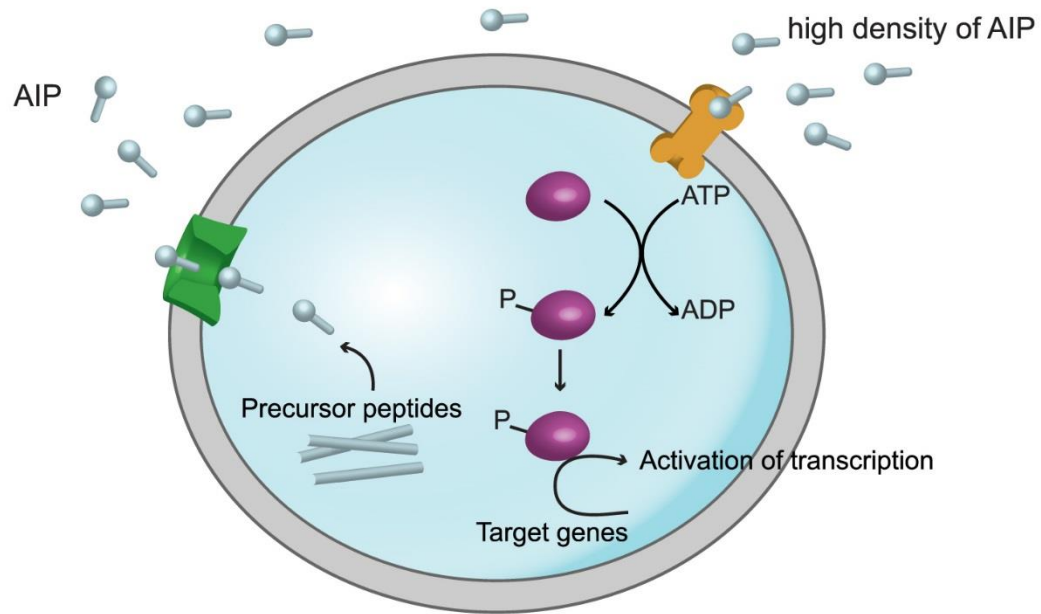
Biofilm Formation

- *Staphylococcus Aureus*: gram-positive coccal bacterium, pathogenic strains produce potent protein toxins and express cell-surface proteins that bind & inactivate antibodies (ie. MRSA)
- *Pseudomonas Aeruginosa*: gram-negative aerobic, coccobacillus bacterium
- Biofilm is composed of extracellular DNA, proteins, and polysaccharides; harder for antibiotic treatment
- Formation via quorum sensing



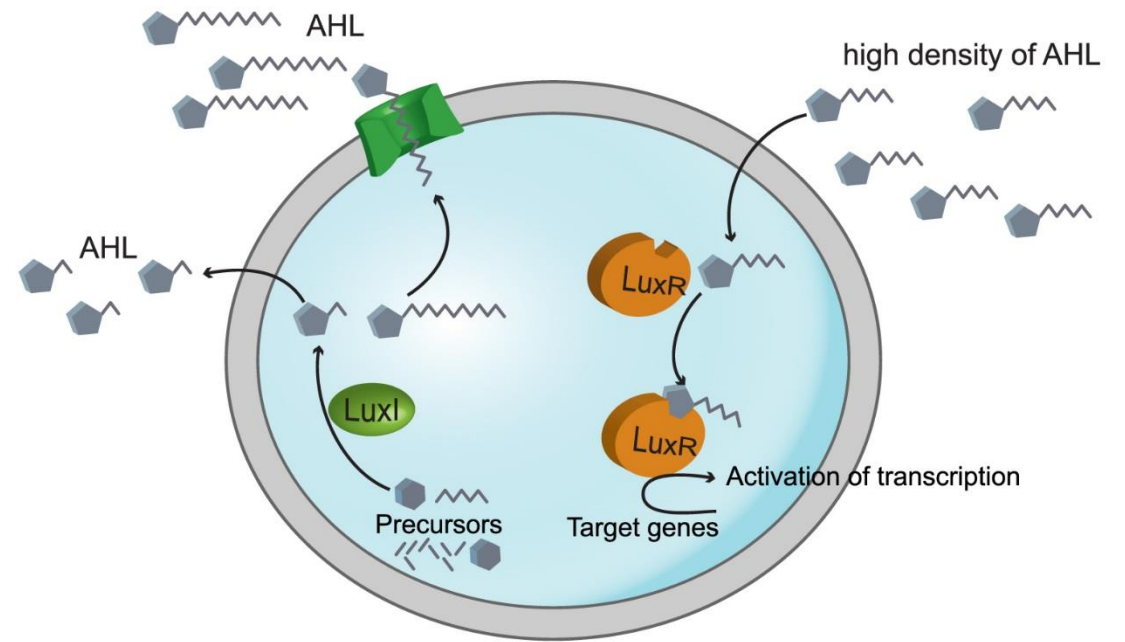
MECHANISM 1

Quorum sensing in mainly Gr⁺ bacteria: autoinducing polypeptides (AIP)



MECHANISM 2

Quorum sensing in Gr⁻ bacteria: N-acylhomoserine lactones (AHL / AI-1)

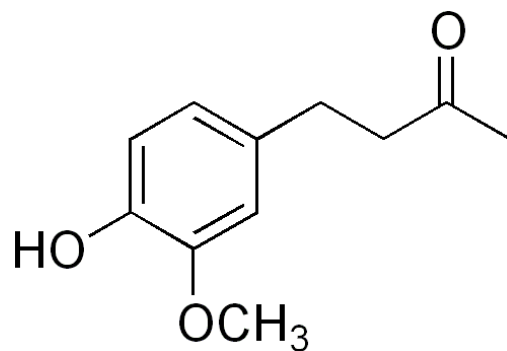


- Quorum sensing is a system of stimulus and response correlated to population density
- Positive feedback loop which depends on detection of inducers produced by neighboring bacterium to activate downstream transcription genes for coordinated behavior



Ginger Extract Inhibits Biofilm Formation

- Inhibit *P. aeruginosa* PA14 biofilm formation through the reduction of c-di-GMP production and consequent reduction of total polysaccharides production via QS inhibition
- Biofilm development was reduced by 39–56% when ginger extract was added to the culture
- Inhibited biofilm formation in both Gram-positive and Gram-negative bacteria
- Zingerone (vanillyl acetone), has been shown to inhibit biofilm formation, to increase the susceptibility of *P. aeruginosa* PAO1 to ciprofloxacin and to inhibit swimming, swarming, and twitching motilities

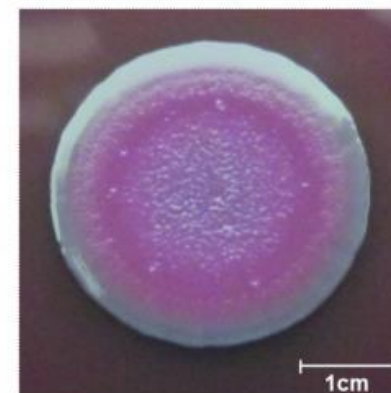


A



Control

B



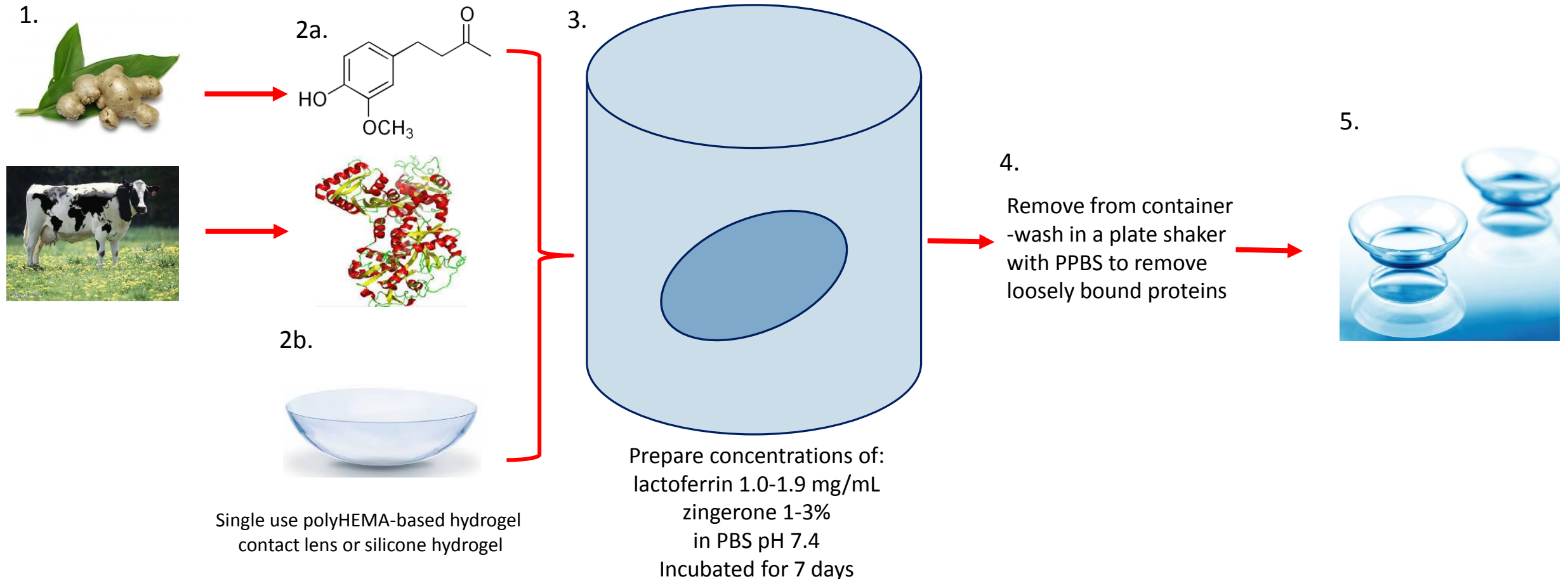
1% Ginger extract

Natural Lactoferrin



- Found in high concentrations in colostrum (first milk) and tear film
- Produced by the acinar cells of the lacrimal gland; immunological defense
- Binds free iron therefore reducing the availability of iron necessary for microbial growth and survival of pathogenesis
- Inhibits biofilm formation
- Binds to pathogen-associated molecular patterns (ie. Lipopolysaccharide – LPS) and removed through tears and hydrodynamic flushing

Anti-Biofilm Networks on Contact Lens



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Pros & Cons

Pros:

- Effective in preventing biofilm formation, corneal epithelial damage, and corneal ulcers
- Biocompatible
- Allows natural, non-pathogenic ocular microbiome to be preserved

Cons:

- Mechanism by which zingerone inhibits poorly understood
- Verification to whether this treatment can be applicable to other types of keratitis infections

Development & Manufacturing



Dr. Mark Willcox
(University of New South Wales)



Questions?



In Development: Antimicrobial Peptide Melamine

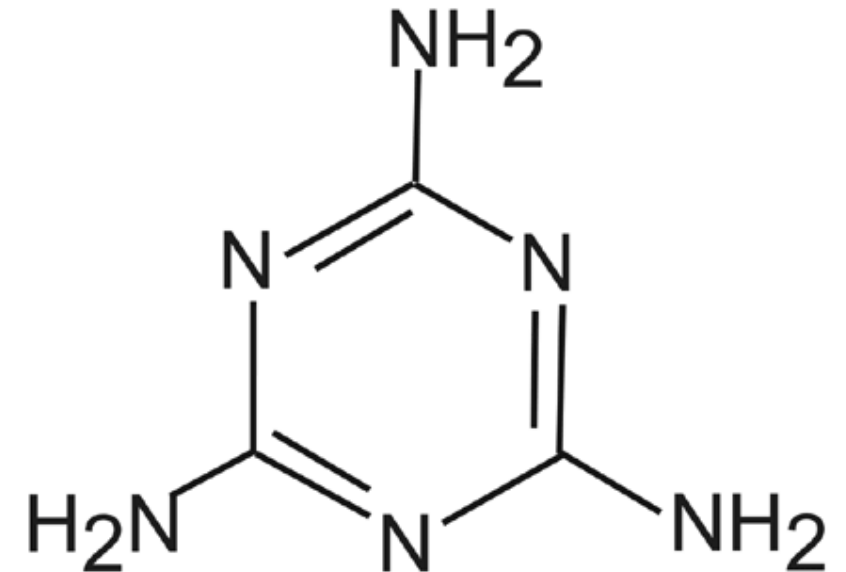
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Pros:

- Effective against many different bacteria, fungi and protozoa
- Not toxic to mammalian cells at active concentrations
- Bacteria do not gain resistance to it

Cons

- Can wipe out all of the naturally occurring ocular microbiome
- Can lead to dysbiosis



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