**Slide Notes**

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The cornea is the transparent part of the eye that covers the front portion of the eye. It covers the pupil (the opening at the center of the eye), iris (the colored part of the eye), and anterior chamber (the fluid-filled inside of the eye). The cornea's main function is to refract, or bend, light. The cornea is responsible for focusing most of the light that enters the eye. The cornea is composed of proteins and cells. The cornea is comprised of five layers: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium.

Bacterial keratitis is an infection of the cornea (the clear, round dome covering the eye's iris and pupil) that causes pain, reduced vision, light sensitivity and tearing or discharge from your eye. Resulting from infection from contact lens use or from injury to the eye, bacterial keratitis usually develops very quickly, and if left untreated, can cause blindness. The bacteria usually responsible for this type of keratitis infection are *Staphylococcus Aureus* and, for contact lens wearers, *Pseudomonas Aeruginosa*.

Common bacterial infections that can cause irritation and redness affect an estimated 7 percent to 25 percent of contact lens-wearers, and much rarer keratitis infections can even cause blindness.

Contact lens wearers particularly are susceptible to eye irritation that can lead to a corneal ulcer. A contact lens may rub against the eye's surface, creating slight damage to the epithelium that may enable bacteria to penetrate the eye.

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If no organisms are identified on the slide smear, initiate broad-spectrum antibiotics with the following: tobramycin (14 mg/mL) 1 drop every hour alternating with fortified cefazolin (50 mg/mL) 1 drop every hour.

Treatment for corneal ulcers and infections depends on the cause. Treatment should be started as soon as possible to prevent scarring of the cornea. If the exact cause is not known, you may be given antibiotic drops that work against many kinds of bacteria. Once the exact cause is known, you may be given drops that treat bacteria, herpes, other viruses, or a fungus. Severe ulcers sometimes require a corneal transplant. Corticosteroid eye drops may be used to reduce swelling and inflammation in certain conditions. Your health care provider may also recommend that you: avoid eye makeup, do not wear contact lenses at all, or do not wear them at night, take pain medications, wear an eye patch to keep out light and help with symptoms, and wear protective glasses

Infections often come when people don’t take proper care of their lenses—sleeping in them overnight, or not cleaning them well or often enough. According to one 2010 study, while 86 percent of contact-wearers thought they did a good job caring for their lenses, only 32 percent showed “good compliance.” Forty-four percent did average, and 24 percent were “noncompliant.”

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About six years ago, Valery Shestopalov of the Bascom Palmer Eye Institute at the University of Miami was speaking with his microbiology colleagues about what bacteria are found on normal, healthy eyes. Conventional wisdom at that time held that healthy eyes don’t harbor much microbial life, tears and blinking tend to clear away foreign objects, including bacteria. But Shestopalov’s early tests revealed something different. “The tests ran positive. All exposed mucosal epithelium are populated densely,” he said. In 2009, Shestopalov began the Ocular Microbiome Project with funds from his institution. Eventually, he secured a grant from the National Eye Institute and began collaborating with Russell Van Gelder at the University of Washington, who had been developing PCR-based diagnostic tests to identify bacteria and fungi on the eye. The project now has a dozen collaborators at five universities.

Shestopalov presented preliminary ocular microbiome data at the Association for Vision Research and Ophthalmology [annual meeting](http://www.arvo.org/Annual_Meeting/) held in Orlando, Florida. His team sequenced samples from healthy corneas, contact lenses, and conjunctiva—the inner surface of the eyelids—using 16s ribosomal RNA sequencing, along with a new method Van Gelder developed called Biome Representational in Silico Karyotyping ([BRiSK](http://genome.cshlp.org/content/21/4/626.long)), which uses high-throughput sequencing to identify bacteria at the species level.    
  
The team found that about a dozen bacteria genera dominated the eye’s conjunctiva, a third of which could not be classified. On the corneal surface, they found a slightly different community. Again, about a dozen genera dominated. And everywhere they’ve looked, the researchers have found more than just bacteria. “We haven't published on this yet, but I have been surprised by how often we find phage or viruses on the normal ocular surface,” said Van Gelder.

In Figure: Relative abundance of bacterial taxa in the conjunctiva. (**A**) Phylum-level representation of the bacteria at the OS of the four subjects calculated according to relative abundance of classified 16S rRNA gene reads. The percentage of reads that failed to classify to known bacterial phyla is indicated as Unclassified, shown in orange. The circular diagram presents average values calculated for all analyzed subjects. Color-coding legend on the right shows taxonomic identities of the classified bacteria. (**B**) Genus-level representation of the bacteria at the OS. Unclassified reads (31% of the total 115,003 sequences) are shown in *dark blue*. (**C**) Relative abundance of known (16S-classified) and novel (unclassified) bacterial phylotypes at the conjunctiva of the individual subjects. All percentages were calculated relative to the total number of qualified DNA reads for each individual.

The researchers also found that during keratitis infections—infections of the cornea—only about half as many bacterial varieties were present, most prominently *Pseudomonas* strains. The changes typically occurred well before a diagnosis of an eye infection, suggesting the ocular microbiome could inform future diagnostics, Shestopalov noted. His team is refining the algorithm for predicting infection based on these changes to the make-up of bacteria and the timing of these changes.  
  
One factor that may be expected to impact the composition of the ocular flora is the use of contact lenses. Contact lens wear is one of the biggest factors leading to corneal infection. Common bacterial infections that can cause irritation and redness affect an estimated 7 percent to 25 percent of contact lens-wearers, and much rarer keratitis infections can even cause blindness. Researchers believe contact lenses make it easier for pathogens to colonize the surface of the eye by giving the bacteria something to adhere to. Sequencing biofilms from used contact lenses, Shestopalov’s team found evidence of microbial communities that were different from the ocular microbiomes of people who don’t use contacts. On the lenses themselves, the researchers have found much less diversity—many of the bacterial genera that dominate the conjunctiva and cornea were depleted. In their place, *Staphylococcus* dominated.

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The development of a biofilm *in vitro*involves the following 5 stages (Stoodley and others 2002):

Stage 1: reversible attachment of bacterial cells to a surface,

Stage 2: irreversible attachment mediated by the formation of exopolymeric material,

Stage 3: formation of microcolonies and the beginning of biofilm maturation,

Stage 4: formation of a mature biofilm with a 3-dimensional structure containing cells packed in clusters with channels between the clusters that allow transport of water and nutrients and waste removal, and

Stage 5: detachment and dispersion of cells from the biofilm and initiation of new biofilm formation; dispersed cells are more similar to planktonic (that is, nonadherent) cells than to mature biofilm cells.

Leads to corneal epithelial damage following biofiolm detachment & dispersion.

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Bacteria that use quorum sensing constitutively produce and secrete certain signaling molecules (called *autoinducers* or *pheromones*). These bacteria also have a receptor that can specifically detect the signaling molecule (inducer). When the inducer binds the receptor, it activates transcription of certain genes, including those for inducer synthesis. There is a low likelihood of a bacterium detecting its own secreted inducer. Thus, in order for gene transcription to be activated, the cell must encounter signaling molecules secreted by other cells in its environment. When only a few other bacteria of the same kind are in the vicinity, diffusion reduces the concentration of the inducer in the surrounding medium to almost zero, so the bacteria produce little inducer. However, as the population grows, the concentration of the inducer passes a threshold, causing more inducer to be synthesized. This forms a positive feedback loop, and the receptor becomes fully activated. Activation of the receptor induces the up-regulation of other specific genes, causing all of the cells to begin transcription at approximately the same time. This coordinated behavior of bacterial cells can be useful in a variety of situations.

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Figure: Comparison of colony morphology on Congon red agar plates. (A) Colony of PA14 grown without ginger extract (B) Colony of PA14 grown with ginger extract.

Preparation of ginger extract Ginger (Zingiber officinale) extract was prepared according to the protocol described previously [37]. Briefly, 150 g ginger root was shredded with 300 mL toluene (99.9%) using a standard kitchen blender. After shredding, debris was allowed to settle for 24 h at room temperature. The supernatant was filtered through a Whatman no. 1 filter paper (pore size = 11 µm). Then 150 mL deionized water was added to 150 mL filtrate, and the mixture was stirred using a magnetic stirrer for 24 h at room temperature. The mixture was then left to form water and toluene phases. The water phase was collected using a pipette and filtered through a 0.22-µm micro filter (Millex® filter, Carl Roth, Karlsruhe, Germany). The filtrate (100% ginger extract) was used to test whether or not ginger extract inhibits biofilm formation.

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Lactoferrin (LF), also known as lactotransferrin (LTF), is a multifunctional protein of the transferrin family. Lactoferrin is a globular glycoprotein with a molecular mass of about 80 kDa that is widely represented in various secretory fluids, such as milk, saliva, tears, and nasal secretions. Lactoferrin is also present in secondary granules of PMN and is secreted by some acinar cells. Lactoferrin can be purified from milk or produced recombinantly. Human colostrum (*"first milk"*) has the highest concentration, followed by human milk, then cow milk (150 mg/L).

Lactoferrin is one of the components of the immune system of the body; it has antimicrobial activity (bacteriocide, fungicide) and is part of the innate defense, mainly at mucoses.In particular, lactoferrin provides antibacterial activity to human infants.Lactoferrin interacts with DNA and RNA, polysaccharides and heparin, and shows some of its biological functions in complexes with these ligands.

The surface of the eye provides an inert barrier against infection. Through its unique combination of antimicrobial action and antiinflammatory activities lactoferrin (Lf) in the tear film plays an important role in the maintenance of ocular health. In order to maintain clarity the eye must provide immunological defense without immunopathology. Along with physical barriers, soluble plasma factors and other proteins such as lysozyme, Lf produced by the acinar cells of the lacrimal gland serves a number of roles in defense for this purpose. Lf in tears provides antimicrobial efficacy by binding free iron thus reducing the availability of iron necessary for microbial growth and survival as well as pathogenesis. Lf has been shown to inhibit biofilm formation and thus may play a role in protecting contact lens surfaces from colonization. Virus particles’ entry into epithelial cells is inhibited by Lf while an excess of Lf in tear film is thought to limit the opportunistic Lf-mediated bridging of adenovirus and host cell that occurs in other tissues. Lf dampens the classical complement activation pathway by binding to markers of inflammation and immune activation while pathogen-associated molecular patterns such as lipopolysaccharide (LPS) are targeted by Lf for removal through tears and hydrodynamic flushing.

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Process:

1. & 2a. Zingerone is extracted and purified from Ginger according to Kim H-S method. Lactoferrin isolated from bovine (due to shared antigen determinants between bovine and human).
2. b. Single use polyHEMA-based hydrogel contact lens or silicone hydrogel is purchased.
3. Incubation period for lactoferrin and zingerone absorption into contact lens.
4. Remove excess
5. Finished product

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Protein Coating on Contact Lenses

 Lenses of each lens type were coated with three different tear proteins: lysozyme, lactoferrin, and albumin (Sigma-Aldrich, Castle Hill, NSW, Australia). Lysozyme, lactoferrin, and albumin solutions were prepared at a concentration of 1.9, 1.9, and 0.5 mg/ml respectively. The lenses were incubated at the specified concentration for the following time period based on the results from previously published studies. The CH lens material was incubated in the lysozyme solution for 5 days, while the SH lens materials were incubated for 7 days. All the lenses were incubated in lactoferrin and albumin solutions for 7 days. After the specified incubation periods, the lenses were removed from the vials and washed in a plate shaker with PBS to remove loosely bound protein. These lenses were the “protein-coated” lenses.

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