Robert Bhisitkul and Tejal Desai Teaching Tools
Prepared by Lindsay Osso & Valentina Garcia (Graduate Division, UCSF)

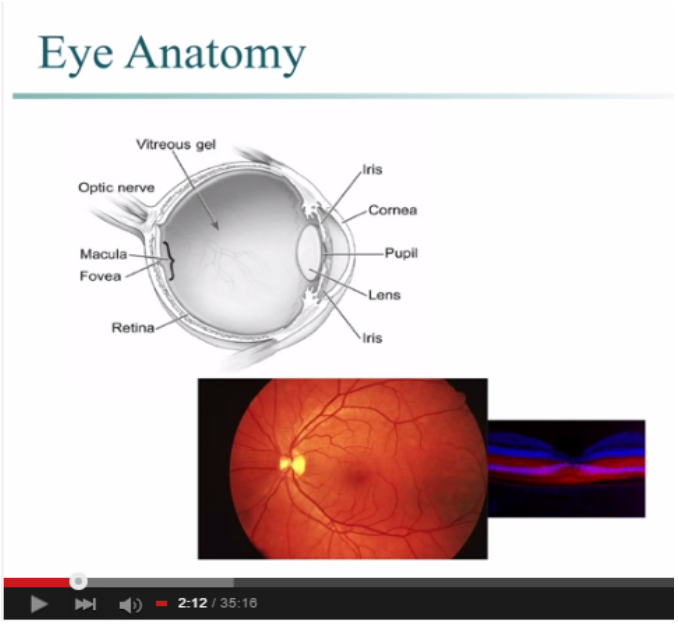
ADVANCING THE TREATMENT OF RETINAL DISEASES

iBiology lectures by Robert Bhisitkul and Tejal Desai (UCSF)

KEY TERMS AND CONCEPTS

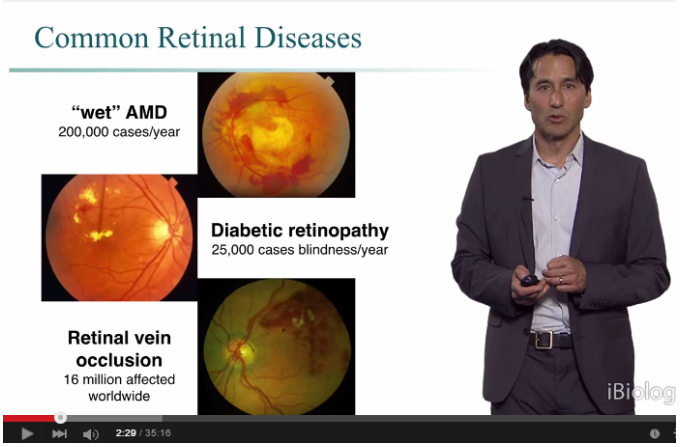
- Wet age-related macular degeneration
- Ocular drug delivery
- Bioengineering
- Diffusion
- Nanopores
- Therapeutic window
- Nanotemplating

LECTURE NOTES



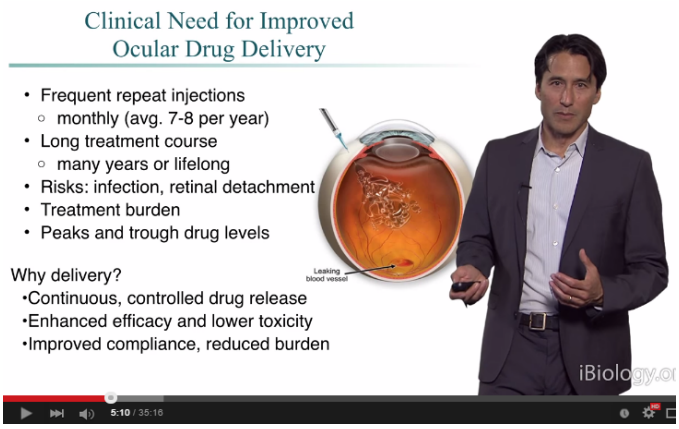
0:56-2:18 Eye anatomy.

Light enters the eye through the *pupil*. Both the *cornea* and the *lens* refract the light to focus it on the *retina.* The retina is the light sensitive neural tissue of the eye; it sends an image to the brain. In the retina, the *fovea* within the *macula* is responsible for the highest resolution vision. The *vitreous gel* is a clear, gel-like substance that fills the space between the lens and the retina, where drugs are injected to be delivered to the retina.



2:19-3:40 Retinal diseases.

Age-related macular degeneration (wet): blood vessels grow under the retina causing blood and fluids to leak into the retina. *Diabetic retinopathy:* blood and fluids leak into the retina, causing blindness. *Retinal vein occlusion:* blocked circulation in back of the eye, leading to hemorrhaging into the retina.

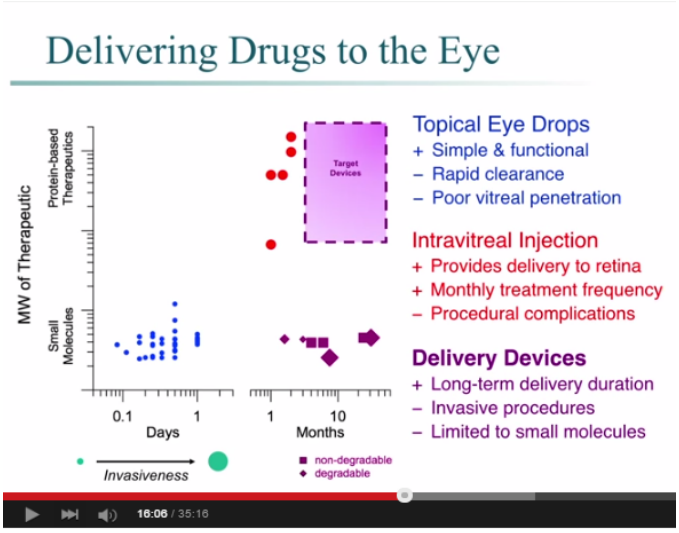


3:41-6:26 Improved ocular drug delivery is needed.

New drugs have been developed to treat many retinal diseases. These drugs are currently delivered to the eye by injection through the cornea. A less invasive and less frequent delivery system is needed.

9:39-11:09 Desired features for an ocular drug delivery device.

Dr. Bhisitkul and Dr. Desai have collaborated together to determine the ideal properties for an ocular drug delivery device.

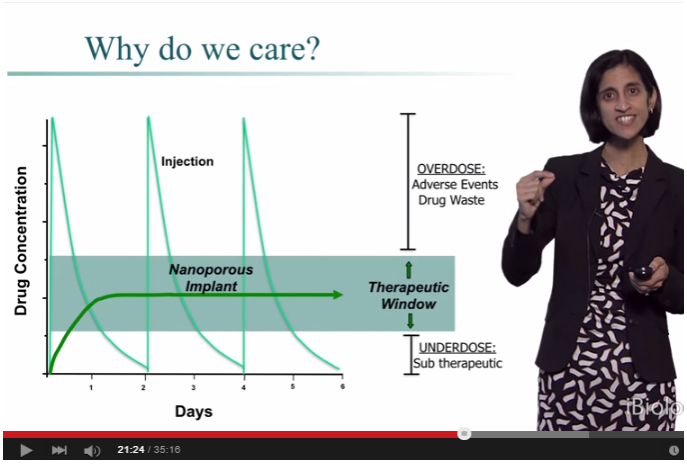


14:18-16:13 Current and future ocular drug delivery techniques.

Dr. Desai outlines the goal for their target drug delivery devices. Importantly, these devices must be long lasting and accommodate large drug molecules. While there are long lasting drug delivery devices currently available, these devices are limited to the delivery of small molecules.

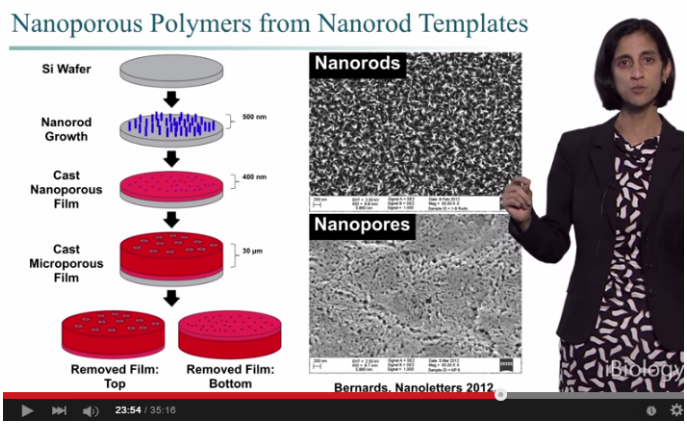
16:14-18:57 Linear diffusion of drugs using nanopores.

By decreasing the size of the pore to 1-2 times the size of the drug contained within the pore, drug molecules can only leave one at a time, allowing for linear diffusion into the surrounding tissue. Increasing the number of nanopores increases dosage.



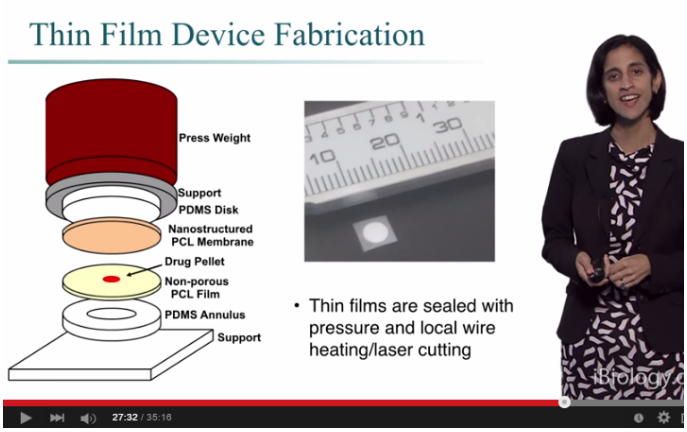
20:59-21:48 Nanopores maintain drug levels in the therapeutic window.

Stable rates of linear diffusion allow for consistent drug levels. This allows drug concentrations to remain in the therapeutic window, where they are effective but non-toxic.



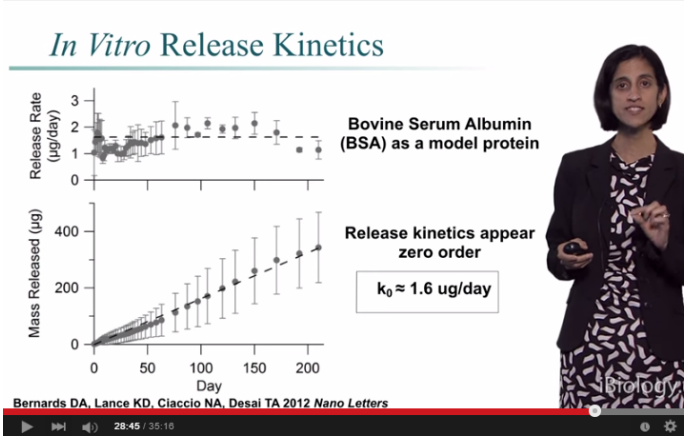
21:49-25:27 Nanotemplating to create nanopores.

To create the nanopores for the drug delivery devices, inorganic nanorods are used as a template. The biodegradable material of choice is cast over the nanorods to create nanopores.



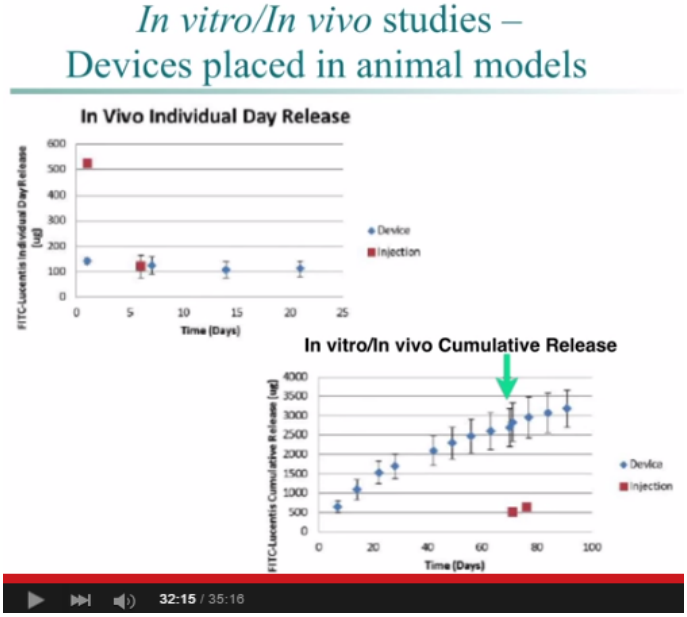
26:45-26:09 Assembly of the drug delivery device.

In the final delivery device, the drug is placed between a porous and a non-porous membrane that are fused together. The drug exits through the nanopores. This device can be rolled up and injected from a needle into the eye.



28:11-29:37 The device releases protein linearly *in vitro*.

In a dish in the laboratory, the drug delivery device successfully maintains a constant release rate for 200 days. They use a model protein BSA rather than a drug.



31:28-32:40 The device releases drug linearly *in vivo*.

In a rabbit eye, the device (blue) releases drug consistently for 22 days (upper left graph). As compared to the injection (red) whose drug concentration drops drastically five days following injection, the device is much more effective at maintaining consistent drug levels between days.

REVIEW QUESTIONS

1. What are the main disadvantages with the current mechanisms of drug delivery to the eye?

Answer: (3:41-6:26)
- Monthly injections over a long period of time
    - High treatment burden for patients
    - High work burden for health care providers
- Risk of infection, retinal detachment
- Inconsistent drug levels

2. What are the desired properties of a new ocular drug delivery device? Why are these properties important?

Answer: (9:39-11:09)
- Long lasting
    - Reduces doctor visits
- Consistent drug levels
    - Maintains optimal treatment dosage – avoids toxicity at high drug levels and sub-therapeutic concentrations at low drug levels
- Similar procedure to current office procedure
    - Reduces training of doctors
    - Low cost
- Biodegradable materials
    - No accumulation of old devices in the eye
- Adaptability for a range of drugs
    - Usability with many drugs for many retinal diseases

3. Why can't drugs that are used to treat diseases of the retina be delivered to the eye through non-invasive eye drops?

Answer: (14:40-15:07)
- Low penetration to the retina
- Limited to small molecules
- Short lasting

4. How does Dr. Desai's drug delivery technology ensure linear drug diffusion?

Answer: (16:14-18:57)
In traditional drug diffusion technology, there is a reservoir for the drug and an opening to the tissue to which the drug is being delivered. The drug exits the reservoir through a physical process known as diffusion. Diffusion occurs due to the random motion of molecules. Sometimes, this random motion will

be in the direction of the exit of the reservoir, causing drug molecules to leave the reservoir and enter the tissue. The chance of a single molecule exiting the reservoir is independent of the other molecules. However, the number of total molecules exiting the reservoir will scale with the number of molecules in the reservoir. Initially, the rate of drug delivery will be high. As more molecules leave the reservoir, there are fewer remaining in the reservoir, so the rate of drug delivery will decrease.
Dr. Desai's drug delivery technique employed the use of nanopores to cause linear diffusion of molecules. The opening out of which the drug can escape is only 1-2 times the size of the molecule of drug. It is called a nanopore. The properties of diffusion still apply: the chance of a single molecule leaving the reservoir is independent of the other molecules. The main difference, however, is that the opening through which the molecules exit can only fit one molecule at a time. Thus, the rate at which molecules leave is only dependent on the rate of diffusion of an individual molecule, and is independent of the concentration of molecules in the reservoir. This allows for a constant rate of diffusion throughout the drug delivery process.

5. How does the release kinetics of the device compare *in vitro* versus *in vivo*?

   Answer:
   In vitro, a sample protein bovine serum albumin (28:11-29:37) is released consistently at 1-2 µg/day for 200 days. Lucentis (29:38-30:11) is released *in vitro* at 2.5µg/day for 120 days. The release of both of these substances is linear over time.
   In vivo, Lucentis (31:28-32:40) is released at 125µg/day over 22 days. The higher release rate may be due to a different device being used – it was not specified in the talk. The release of Lucentis *in vivo* also remains linear over this 22 day period.

DISCUSSION QUESTIONS

1. Lucentis is a drug used to treat wet age-related macular degeneration. It is an antibody fragment against VEGF. What is VEGF? From what Dr. Bhisitkul mentioned about wet age-related macular degeneration, how do you think that blocking VEGF would help treat this disease?

   Answer: (6:55)
   VEGF (vascular endothelial growth factor) is protein secreted by cells that stimulates the formation of blood vessels. An antibody against VEGF would block its function, thus preventing VEGF from stimulating the blood vessel formation. In wet AMD, there is ectopic growth of blood vessels under the retina that eventually leak, causing the symptoms of wet AMD. Thus, preventing the formation of new blood vessels helps treat this disease.

2. Dr. Bhisitkul discusses the phase three clinical trial of Lucentis (7:08). What is a phase three clinical trial? What are the other phases of clinical trials?

   Answer:
   There are four phases of clinical trials that are undertaken in the process of getting a new drug on the market. The first phase is to test for toxicity of and tolerance to the drug in a small group (tens) of individuals. Phase two requires a larger group (hundreds); it tests for toxicity, dosage, and efficacy. Phase three involves yet a larger group than phase two (hundreds to thousands) and assesses the efficacy of the drug compared to the current treatment standards. After demonstrating efficacy and safety of a drug, it can be marketed. The fourth and final phase basically involves monitoring patients on the drug over a longer period of time. If safety issues arise in this period, the drug may be taken of the market or its use may be restricted.

3. Dr. Desai shows that her drug delivery device can release drug more consistently than drug injections in rabbits, maintaining drug concentrations in the therapeutic window for longer periods of time. What are some next steps that should be taken to show that this method of drug delivery is more beneficial for patients?

   Answer:
   In an animal model of wet age-related macular degeneration, they should compare the disease progression of three groups: (1) no treatment, (2) current drug injection regime, (3) drug delivery device injection. Despite the fact that this drug delivery device releases drug at a more consistent rate than the current drug injection regime, it is still important to test its efficacy. This is for various reasons. There could be unpredicted side effects of the device. It is unlikely, but the consistency of drug delivery may not be ideal to treat this disease.
   Following testing this device in an animal model, the device should be tested on wet age-related macular degeneration patients before approval for general use (see the answer to question 2 for how this process works). Not only should the device be tested for efficacy in patients, but it should be tested to see if it meets the other targets of the device, such as improving patient compliance, improving patient comfort, decreasing the frequency of injections, and successfully degrading in the eye.

4. What are some possible safety concerns with Dr. Desai's drug delivery device?

   Answer:
   This question is intended to be open-ended. Many different answers are welcome. An example of a possible answer follows.

The drug is stored in a reservoir and slowly is released through nanopores. However, it is possible that the device could rupture or degrade prematurely (it is biodegradable) and the drug could all be released at one time. This could cause the drug concentration in the eye to reach toxic levels, which could cause harm to the eye. From 31:28-32:40, it can be seen that the amount total drug stored in the device (cumulative release in blue) is much higher than that of one injection (red), meaning that a leak could cause drug concentrations in the eye to be much higher than they are in the current injection paradigm.

5. The drug delivery device was designed so that it could be used in various situations. What are some other possible applications of this drug delivery technology in other body systems?

   Answer:
   This question is similarly open-ended. Below is an idea.
   Medications that are taken orally daily may be more effective with an injection of this device filled with drug to the targeted area of the body. Firstly, this would decrease the drug amounts required as the drug would not have to pass through the digestive system. This would also decrease systemic side effects that the drug has on non-target body areas. Finally, the patient would no longer be responsible for taking their pills each day, which may increase patient compliance and overall treatment outcomes.