

How I Spent My Summer Rotation

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Interests: capillary permeability

If we can understand the mechanisms behind leaky capillaries,
can we learn how to shut them off?

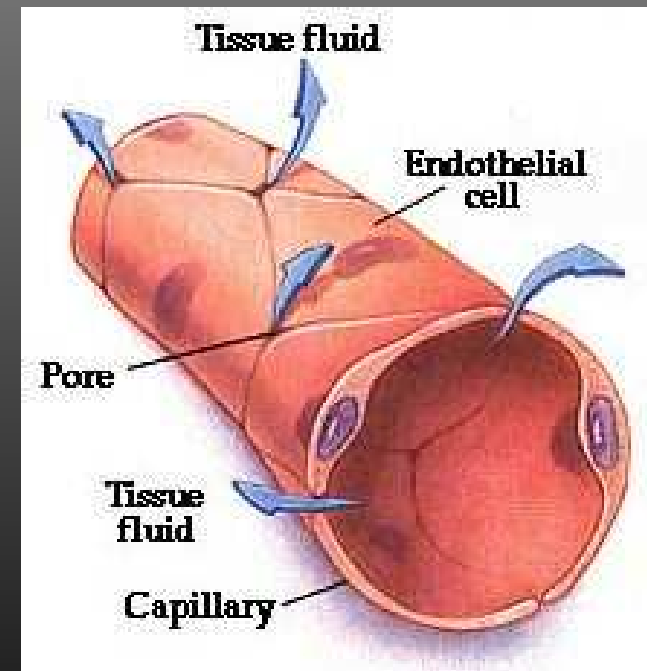


Why Am I Interested?

- exchanges between blood and tissues occur in capillaries
- permeability can be increased by shear stress and transvascular pressure (linked to mechanotransduction and glycocalyx)

shear stress → glycocalyx
→ internal NO mechanism
→ increased permeability

increased pressure → glycocalyx
→ internal NO mechanism
→ increased permeability



Capillary Permeability

- fluid movement through capillary walls involves hydrostatic and oncotic forces
- Starling's equation:

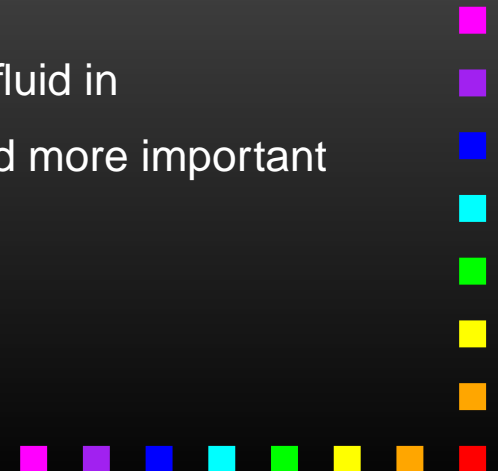
$$J_v = L_p[(P_c - P_i) - (\Pi_c - \Pi_i)]$$

J_v Net movement, L_p Hydraulic conductivity

$P_{c,i}$ Capillary, interstitial hydrostatic pressure

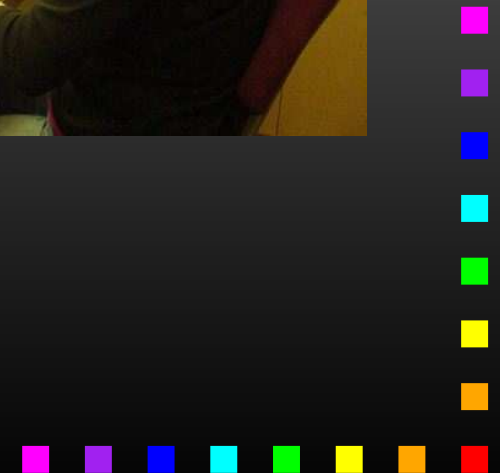
$\Pi_{c,i}$ Capillary, interstitial oncotic pressure

- hydrostatic pressure pushes fluid out, oncotic pressure pulls fluid in
- pressure difference across **glycocalyx** is becoming more and more important (Weinbaum et al. 1999)



How can I measure pressure effects in the lab?

- Learn proper cell culture techniques
- Prepare a "good" monolayer of cells
- Set up experiments properly using the *bubble tracker*
- Measure net fluid flow through monolayers for different pressures



Cell Culture

know your sterile technique!

Lunch time for the cells
Note: feed regularly



Keep the cells/food nice and warm
Hope to recreate a natural environment



Making Monolayers - Plating Cells

- Cleave cells from flasks using trypsin
- Check under microscope to make sure they are floating

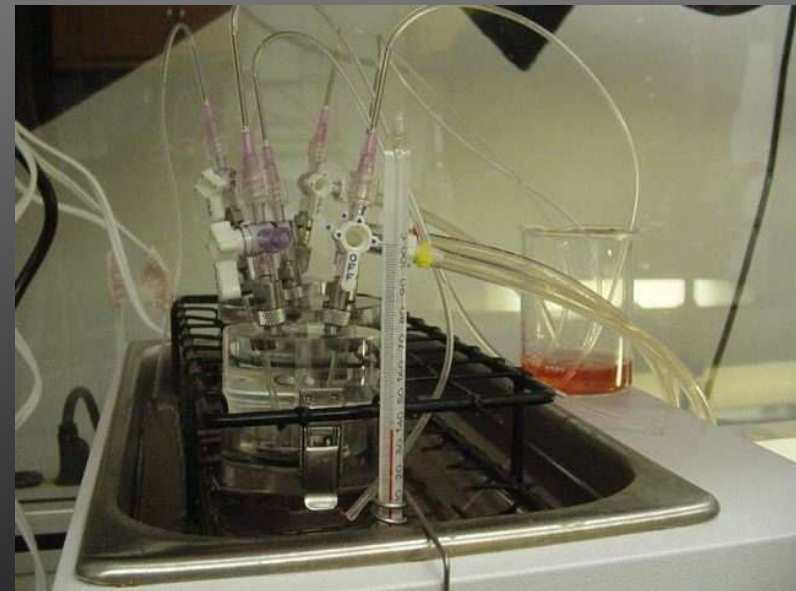


- Warm up gelatin and fibronectin covered polycarbonate filters placed in wells
- Add cells with CARE!

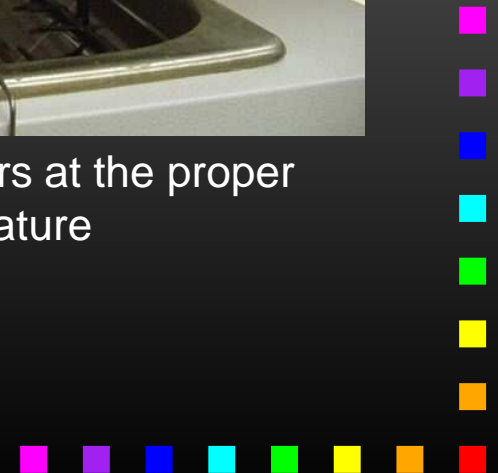


Putting Monolayers in Pressure Chambers

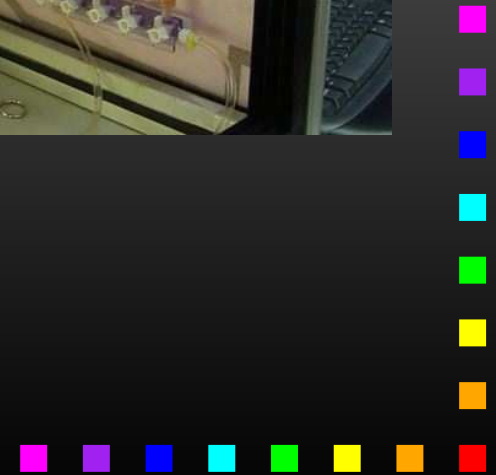
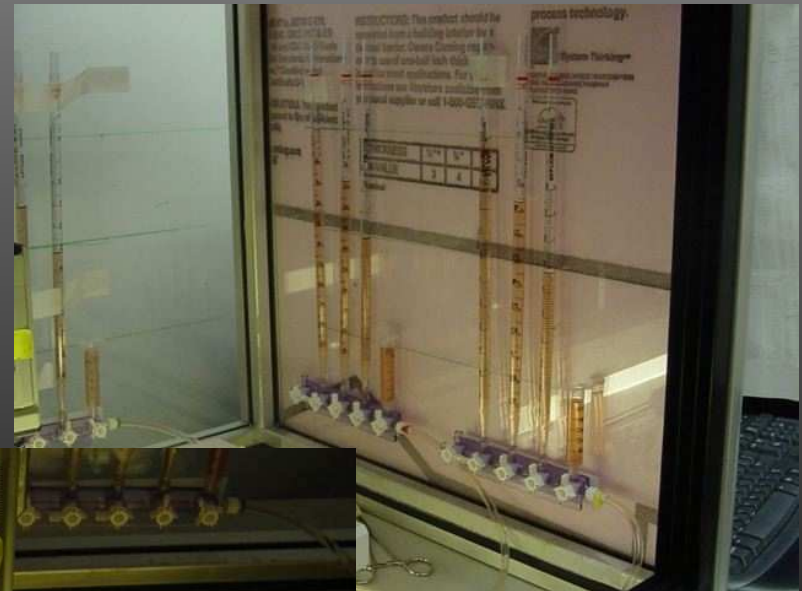
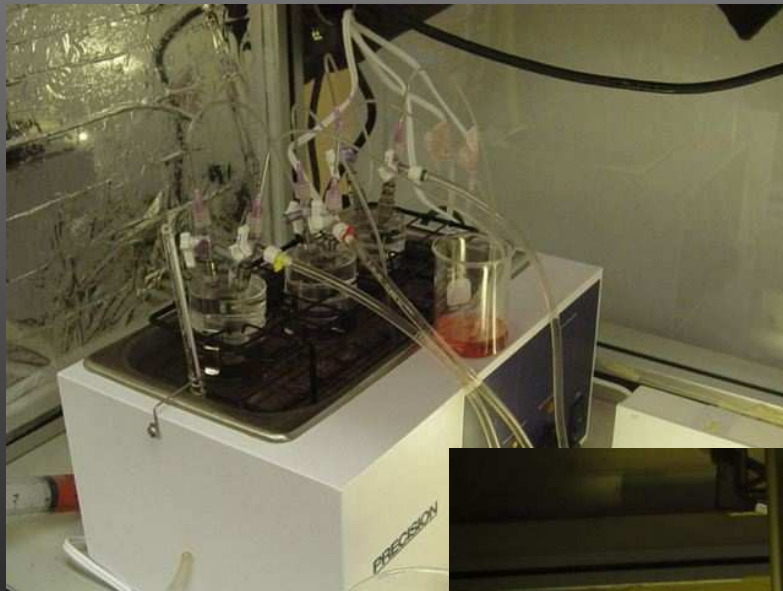
Remove any air bubbles from chambers



Keep the chambers at the proper temperature

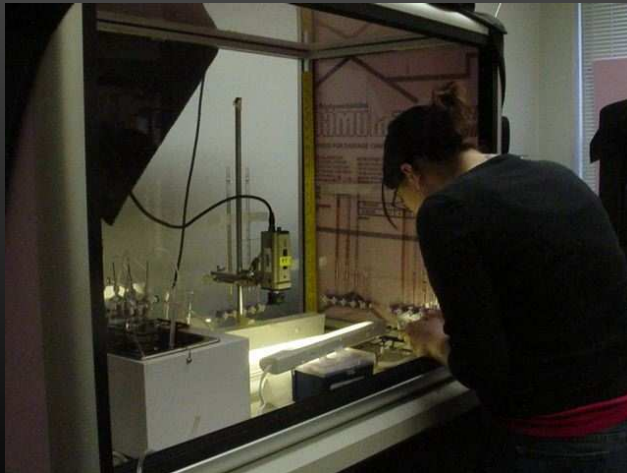


Bubble Tracker



What I learned

- to practice proper cell culture, one must be CAREFUL
- keeping unwanted bubbles out of the tubes is TRICKY
- making wanted bubbles the right size takes PRACTICE
- figuring out 21 valves and the direction the fluid is supposed to go is WEIRD
- making sure fluid doesn't spill all over the place is ANOTHER STORY



BIOLOGY IS HARD!!!

THANKS: Randy, Scott and Ian
for letting me screw up experiments and
not getting mad

Also thanks to IGERT and all the people
involved!

