

Chip Preparation

- 1) Gather an ice bath, conical tubes, collagen type I, Magnesium Chloride, Calcium Chloride, 1ml, 5ml, & 10ml syringes, PDMS microchips, petri dishes and aluminum foil
- 2) Remove the tape from the PDMS microchip and plasma treat using protocol 2 on a petri dish covered by aluminum foil
- 3) Create a solution with a concentration of 100ug/ml collagen type I diluted in PBS (-/-) and place it in a conical tube and in the ice bath
- 4) Create a solution of 750ul of $MgCl_2$, 1ml of $CaCl_2$ and 8.25ml of deionized water into another conical tube and then an ice bath
- 5) Use a 1ml syringe to push the collagen solution through the PDMS microchannels until the solution comes out the other end and there are no more bubbles present in the channels
- 6) Plug the holes of the PDMS microchip using cut off ends of 5ml syringes (possibly to be replaced with 3D printed models in the future)
- 7) Place the microchip in a petri dish with a PBS bath with the lid closed and incubate at 37 degrees Celsius for about 1 hour

Vessel Simulation

- 1) Create cannulas out of 5ml syringes
- 2) Mix 18ul of fibrogen and 10ul of calcium with 6ml of patient blood.
- 3) set a syringe of 5ml of PBS into a dosing pump set at a pulling flow rate of 70ul/min
- 4) Connect tubing from the PDMS syringe to one of the chip's ends
- 5) Attach one of the previously made cannulas to the other end of the chip
- 6) Connect a pressure sensor to the PDMS syringe
- 7) Properly secure the chip to the microscope to be observed
- 8) Boot up the camera of the microscope and run some test images
- 8) Dispense 1ml of blood into the chip via its canula
- 9) After a few minutes, dispense 100ul of the previously made $MgCl_2$, $CaCl_2$, and water solution
- 10) Ideally, observe a dip in pressure right before the blood sample reaches the syringe
- 11) Dispose of blood chip, tubing, and other excess material and turn off the microscope

Taking Images

- 1) Open "Gen 5 3.10"
- 2) Pick "Imager Manual Mode"

- 3) Press "Capture New"
- 4) Select "All" under "Filter"
- 5) Select "Abidemi" for vessel type
- 6) Change the image mode to "Phase contrast" located at the top left of the page
- 7) Got to the highest point of the section of the channel being imaged
- 8) Press "imaging mode" and check "montage"
- 9) Press "set top"
- 10) Go to the lowest point of the section of the channel being imaged and press "set bottom"
- 11) Go to the right-most point of the section of the channel being imaged and press "set right"
- 12) Go to the left-most point of the section of the channel being imaged and press "set left"
- 9) check "kinetic imaging"
- 10) click "edit imaging step"
- 11) click "start kinetic"