Flow Channel Protocol

Wednesday, June 26, 2019 3:01 PM

Note: "FPGA Script:" means to run the script on the MATLAB instance that controls the FPGA board, along with the power supply and velmex. "Verasonics Script:" means to run the following script on the MATLAB instance controlling the verasonics

First thing in the morning

Tank

- [x] Fill tank
- [x] Plug in heating elements (orange plug into temperature controller heat outlet)
- [x] Attach lumen to flow channel
- [x] Degass for 2 hours

Plasma and rt-PA

- [x] Get ice from physics therapy
- [x] Get plasma from -80°C freezer, as many tubes as clots planned for the day.
- [x] Put in 37°C water bath to thaw.
- [x] Get rt-PA vials for number of experiments including rt-PA. Get one extra rt-PA vial since some of the aliquots are a bit low. This ensures we can always draw the required 80.4 uL.
- [x] Thaw rt-PA vials by putting them in the water path for a minute, they will thaw quickly.
- [x] Put thawed rt-PA vials on ice until needed
- [x] Once plasma has become a slush and can be poured out, empty plasma into a beaker that has been triple rinsed with nanopure water.
- [x] Put plasma beaker into the water bath and add weigh donuts to prevent it from floating
- [x] Cover lightly with plastic wrap to prevent foreign particles from falling in. Allow air to get to the plasma though, it needs to equilibrate with atmosphere
- [x] Let plasma equilibrate to atmosphere for 2 hours

Note: the rt-PA can be thawed later as well, but the plasma needs to equilibrate for 2 hours

Transducer and Imaging Array

- [x] After tank has started degassing, visually inspect transducer to make sure it's level, if not use level to make it so.
- [x] Cover imaging array with latex cover and place loosely in the aperture of the transducer, no need for alignment yet
- [x] Check for bubbles on face of transducer and imaging array. Use syringe to draw out any extra bubbles

Other things to prep

- [ ] Fill a small beaker with 10 mL*(# of clots) of PBS -- need to dilute from 10x to 1x
- [x] 50 mL syringe for plasma
- [x] Bottles of nanopure water and bleach are filled up

Experiment Prep

- [ ] If doing flow cytometry, prepare 600 μL and 1.5 mL microcentrifuge tubes, one for each clot. In the 600 μL tubes, add 50 mg of aminocaproic acid. Label with clot number (50 mg per 500 uL of perfusate). I like to use the plastic bag from the imaging probe to store these in. **THESE ARE FOR FLOW**

CYTOMETRY

- [ ] For each clot prepare 1.5 mL microcentrifuge tubes for d-dimer measurements. In the 1.5 mL tubes, add 100 mg of aminocaproic acid. Label with clot number. **THESE ARE FOR D-DIMER**
- [ ] Prepare a weigh boat on the scale for clot measurements

Finding the Focus

After tank has degassed

- [x] Check the underside of the transducer, remove any air bubbles using a bend twisty tie by dragging them towards the center
- [x] Verasonics Script: c/users/verasonics/vantage-4.0.0-1812011200/**SetUpL11_5v_128RyLns_Guides**
  set guides to 0 and 55 mm
- [x] ? FPGA Script: FPGA/FPGA_MATLAB/Current_Driver_Functions/**FPGA_Parameters_Velmex**
  set to 40 PRF, 1 cycle, 1.5 MHz. Pulses doesn't matter.
  Make sure velmex is plugged in and thorlabs is unplugged!
  EXTRA NOTE: changed to **FPGA_Parameter_BubblyCreek_Velmex**
- [x] FPGA Script: Start_TXC_Velmex
- [x] Move the imaging array until the focus is aligned with the guide lines at 0, 55 mm. Tighten the screws to hold it in place. Essentially just a sanity check to make sure it all works.
- [x] Close verasonics script
- [x] ? Verasonics Script: ViktorScripts/**SetUpL11_5v_128RyLns_Ablation**
  Has to be run on Vantage folder. Changed to **SetUpL11_5v_128RyLns_Ablation_BubblyCreek**
- [x] ? Click on "Focus" and set the focus.
- [x] Click "Save Positions"
- [x] FPGA Script: Stop_TXC_Velmex
- [x] Set voltage to 0

Clot Preparation

- [ ] Tools needed: Tweezers, scalpel, side-cutters, ruler, cutting board
- [ ] Unlock room with balance
- [ ] Remove clot from pipette by cutting sealed end with side cutters over sink, such that the broken end falls into the sink. Let clot slide into Petri dish along with serum.
- [ ] Cut the clot to 1 cm length, aiming for a uniform piece from the center
- [ ] Take cutting board with petri dish and clot over to the scale. Keep one hand on the petri dish as it slides easily
- [ ] Blot the clot with a kimwipe by placing the clot on the kimwipe and then folding the wipe over the clot. Run the tweezers along the length of the clot to gently blot the entire clot.
- [ ] Using tweezers, place clot in weigh boat and record weight of the clot
- [ ] Remove lumen from flow channel by disconnecting the red connector first, careful not to bend the latex tubing and avoid breaking off the nylon rod. Disconnect black connector
- [ ] Using tweezers place clot in lumen (latex tubing). There is one end with a nylon rod, remove the other end. The one with the nylon rod is marked by a black line on top.
- [ ] Attach lumen to the flow channel and lower stage into the water. Attach black connector first, then red one. Again, careful not to bend the lumen.
- [ ] Angle the stage such that the right hand side is as low as possible, there should be a stop, and the left hand side is as high as possible. Angling the stage like this prevents bubbles from getting trapped inside the lumen when the plasma is drawn through later.
- [ ] Ensure draw syringe is attached to flow loop
- [ ] Add 30 mL of plasma to the reservoir syringe, monitor temperature until it reaches at least $36°C$
- [ ] If using rt-PA, add it now and mix with plasma using standard pipette mixing protocols

Priming the flow channel

- [ ] At a rate of 10 mL/min pull plasma into the flow channel using the syringe pump until the plasma just passes the lumen
- [ ] FPGA: velmex.gui to get the velmex positioning going
- [ ] align the imaging array such that the imaging plane is parallel to the length of the clot
- [ ] Ensure lumen is level and that no bubbles are present.
- [ ] Ensure clot is butted up against the nylon rod. If not, use short draws at 60 mL/min to try to move it or manually draw the syringe. Try to limit amount of plasma drawn in this process. If necessary, push some plasma back at a slow rate (10 mL/min)

Pre-treatment Planning

- [ ] Verasonics Script: SetUpL11_5v_128RyLns_Ablation
- [ ] FPGA: AblationScanner_CrossSection_BubblyCreek
  - [ ] Define your parameters such as PRF, Cycles, etc, as well as patient info
  - [ ] Run the first few cells by pressing CTRL+Enter to evaluate the current cell
  - [ ] Run until you have set the voltage and set up test pulses

Test Pulses

- [x] On the velmex gui, press clear positions
- [ ] Move to the beginning of the clot (right side when looking at the flow channel head on)
- [ ] Zero out x,y,z in velmex
- [ ] Using the velmex positioning system, assess the cavitation at three spots, 0, 5, and 10 mm from the upstream side of the clot. At each location run on the FPGA matlab: AutoPulse_Velmex(PRF, Test_Pulses) for short test pulses. The major criteria is to avoid cavitation outside of the lumen. After that, try to get the cavitation activity as well distributed throughout the lumen as possible.
- [ ] At each location, press Save Position on the velmex gui to save that position for the treatment planning

- [ ] Move to middle of the clot (5 mm) and save image using L11DesPlainesAblation screen. This also forces the velmex motors to move back to the middle at the end of treatment, an audible cue that treatment is complete

Treatment

- [ ] Close the SetUpL11_5v_128RyLns_Ablation window
- [ ] Run entirety of the AblationScanner_CrossSection script. Wait for "Waiting for verasonics…" message
- [ ] Set and run syringe pump at 0.65 mL/min. Wait for meniscus to start moving
- [ ] Verasonics: CHANGED TO **RunL11_5vFlash_PCI_Sync_BubblyCreek** (old = RunL11_5vFlash_PCI_Sync_FlowChannel). This will automatically
- [ ] Monitor for missed triggers and off-target cavitation. If off-target cavitation is present, adjust in situ with velmex positioner control

Post Treatment

- [ ] Raise lumen structure above water as high as possible. Keep lumen flat to prevent it from moving downstream during draining.
- [ ] Pull remaining plasma at 10 mL/min using the syringe pump
- [ ] If collecting perfusate for further analysis, use a small clean beaker to collect perfusate by opening valve by the syringe pump. If not, dump directly into waste beaker
- [ ] Disconnect lumen and remove clot by pushing small amount of saline using a 10mL syringe
- [ ] Weigh clot

D-Dimer

- [ ] Add 1 mL of perfusate to labeled centrifuge tubes primed with aminocaproic acid

Hemolysis

- [ ] Add 1.3 mL of perfusate to labeled centrifuge tubes

Flow Cytometry

- [ ] Add 500 μL of the perfusate using a pipette to the prepared microcentrifuge tube.

Histology

- [ ] Cut 2-3mm section from the center of the clot. Add to the cassette, if possible end side down. Add 5 mL of low gelling agarose around it. The low gelling agarose (2%) is best stored in a small 50 mL flask with a waterproof screw lid on it (orange caps), and submerged in the water bath alongside the plasma. This keeps it from gelling, but also cold enough to not damage the clot.

End of day

- [ ] Turn off heater!
- [ ] Empty tank