NVU Chip Protocol

1. HESFM Media: + bFGF 250ng/ml (HEAT STABLE) + EGF (500ng/ml) + Fibronectin (25ug/ml) + VEGF (250ng/ml) + TB4 (250ng/ml)
2. 1% BSA (Sterile): Discharge all materials (tips, tubes, filter mesh)
3. 5% Gel: 50mg in 1ml of Complete HESFM Media
4. 10% mTG: 100mg in 1ml PBS (going to use at most 60ul of stock to 1ml of 5% Gel)
5. Sterilize Chips, tubing, new tips
6. Use 17-18ul of hydrogel/tissue/mtg per chip in the center channel

Total objects to be discharged with BSA

1. 1ml pipette tips: 4 tips + 1 bonus = 5
2. 200ul Tips: 3 tips + 1 bonus = 4
3. E1-ClipTips: 1 + 1 bonus = 2
4. 100um Filter: 1
5. 1ml Tubes: 1 + 1 bonus = 2
6. 24 well plate (FBS a single well)

Prep

1. Soak filters, tips, vials, plates in BSA to discharge surfaces
2. Reconstitute Gel and mTG, keep warm at 37°C
3. Sterilize Surgical tools and filters (Autoclave)
4. Make 20ml of Complete HESFM (Stable for 1 month at 2°C)

Embedding Protocol

1. Thaw human tissue vial in bead bath. Continue until 50% thawed
2. Transfer tissue to pre-discharged 1ml vial, fill with **cold HESFM (not complete HESFM)**. Using a pre-discharged 1ml pipette, triturate up and down to break down the brain tissue. Once no resistance is felt, switch to a pre-discharged 200ul tip and triturate up and down until no more resistance.
3. Spin the 1ml tube down for 5 minutes at 400rcf.
4. During this part figure out the total volume of hydrogel needed for the chips.
5. When the 5 minutes is up, use a 200ul pre-discharged pipette tip and aspirate the supernatant. **Be careful not to aspirate the vasculature.**
6. Reconstitute the vasculature in 1ml of **cold HESFM** using a 1ml pre-discharged pipette.Quickly transfer the material to the presoaked 100um filter in the 50ml conical tube or side arm flask.
7. Then, pour PBS over the filter (about 25ml total) to filter the small cells out. **Be careful to not overflow the filter! The PBS can slowly drain and its easy to overflow.**
8. Carefully remove the filter from the 3D printed mesh device. Use tweezers to remove.
9. Place the filter in a ultra-low attachment 6-well plate. Fill with 1ml of **cold HESF**. Using the sterile, flat razor blade ([link](https://www.mscdirect.com/product/details/19538909?cid=ppc-google-&mkwid=%7Cdc&pcrid=&rd=k&product_id=19538909&gad_source=1&gclid=Cj0KCQjww5u2BhDeARIsALBuLnOH0Z1aZw7wPVUXgTn1dwJESAEZ5YNTubJw9u_zzfkgHNjCt6ZqeMYaAp8gEALw_wcB&gclsrc=aw.ds)), gently scrape the mesh to dislodge the vasculature from the mesh. Transfer the 1ml of cold HESF to a ultra-low attachment 2ml conical tube and centrifuge at 400 for 5 minutes. After 5 minutes, aspirate the supernatant, and repeat the scraping again if needed.
10. Add 15ul of warmed mTG to the purified vasculature and 250ul of warmed GelCad. Thoroughly mix without creating bubbles.
11. To inject in the microfluidic chips, first plug the in and outlet of the flanking perfusion channels. Then, select the correct program on the E1 autopipette. Fill chips (that are in the holder) on one side only with automatic pipette (~17-18ul per channel)
12. Use remaining hydrogel for control no flow experiments in a 24 well plate
13. Let crosslink for several hours. 4 hours minimum, 18 hours maximum, **do not let the hydrogel dehydrate**.
14. (For the plates) Add full HESFM media, to the plates. Change every 4 days (75% exchange)
15. (For the chips) assemble perfusion bioreactor. Pre-charge all tubing and chip channels with media. Start perfusion. Change media every 4 days (75% exchange).

bFGF -HS Fisher PHG0367

5 x 5ug 🡪 Add 1mL of water to one 5ug vial. Add all 1ml of the solution to 17.75 ml of Complete media. [250ng/ml]

Store Aliquots in -20°C

EGF Peprotech AF-100-15-1MG

5 x 1mg 🡪 Add 1ml of water to one 1mg vial. Add 10ul of the stock to 17.75 ml of complete media. [500ng/ml]

Store Aliquots in -20°C

Fibronectin Thermo 33016015

1 x 5mg 🡪 Add 5ml of water to the entire vial. Add 500ul of stock to 17.75 ml of complete media. [25ug/ml]

Store Aliquots in -20°C

VEGF Peprotech 100-20

5 x 10ug 🡪 Add 1ml of water to one 10ug vial. Add 500ul of stock to 17.75 ml of complete media. [250ng/ml]

Store Aliquots in -20°C

TB4 Peprotech 140-14

5 x 20ug 🡪 Add 1ml of water to one 20ug vial. Add 250ul of stock to 17.75 ml of complete media. [250ng/ml]

Store Aliquots in -20°C

**To make Complete Media:**

Add 17.75 ml of HESFM, then add 1ml of bFGF, 10ul of EGF, 500ul of Fibronectin, 500ul of VEGF, and 250ul of TB4