

Isakson Lab Vascular Cell Co-Culture (VCCC)

1. Coat underside of the Transwells (polyester, 0.4 μm pore diameter; Corning) with Fibronectin (0.1 mg/ml fibronectin stock into 1X HBSS)
2. Invert the transwells into p600 dish with autoclaved water + 1% fungizone. Remove fibronectin and seed each transwell with 7.5×10^4 SMC in media volume of 500 μl per transwell. Incubate at 37°C for 72 hrs, change water every day and media as needed.
3. Add 1.25 mL media to the wells of a 6-well plate. Remove media from the inverted transwells and flip the transwells into the 6-well plate (SMC monolayer facing down in the dish) and coat exposed membrane with 1% gelatin for 1 hr @ 37°C incubator.
4. Remove gelatin and seed each transwell with 3.6×10^5 endothelial cells in media volume of 750 μl . Incubate for 72 hrs @ 37°C incubator, change media daily or as needed.

SMC Media:

M199

10% FBS

1% L-Glut

1% Pen/Strep

EC Media:

M199

10% FBS

1% L-Glut

1% Pen/Strep

20 $\mu\text{g}/\mu\text{l}$ ECGS