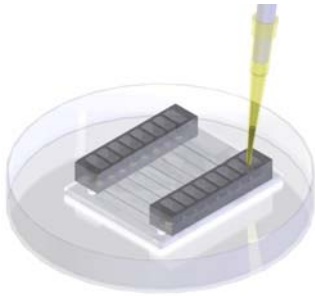


KIMA™ Pump perfusion Protocol using iKIMA App

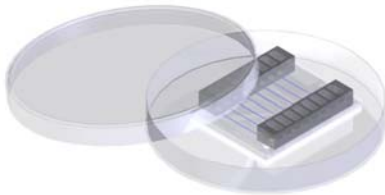
Protocol for Coating and Cell seeding in Vena8 Endothelial+™ Biochips and perfusion using KIMA pump

Step 1



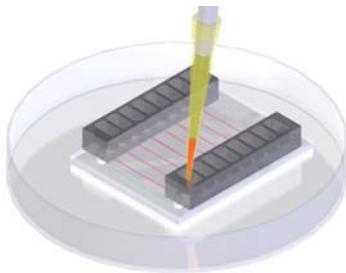
Cellix's Vena8 Endothelial+™ biochips are coated using a standard pipette tip. Dispense ~12 μL of protein (e.g. Fibronectin) into each microchannel. Note the excess of liquid on the entrance and exit ports.

Step 2



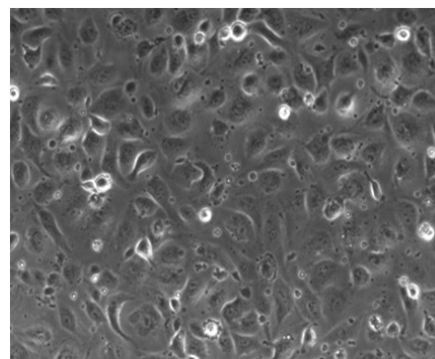
The Vena8 Endothelial+™ biochip is then placed in a humidified sterile petri dish, which should be placed at 4°C for overnight coating.

Step 3



a) After the incubation period, add ~5 μL of 2×10^6 per 100 μl (i.e. 20×10^6 cells/mL)* of endothelial cells gently into each channel.
*Note: concentration specified is for primary HUVEC.

b) The biochip is kept in a sterile petri dish and in the CO₂ incubator for 15 – 20 minutes. Observe the biochip under microscope and top up all the reservoirs with 30 μL of media. Keep the biochip for 1.5 - 2 hrs in the CO₂ incubator.



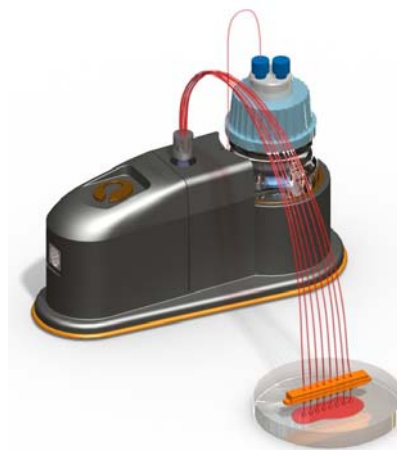
HUVEC primary cells seeded in Vena8 Endothelial+ biochip microchannel: 1.5hrs post-incubation.

Step 4



Wash Kima pump with 70% ethanol and then with sterile distilled H₂O using a 5mL sterile syringe in the biosafety hood.

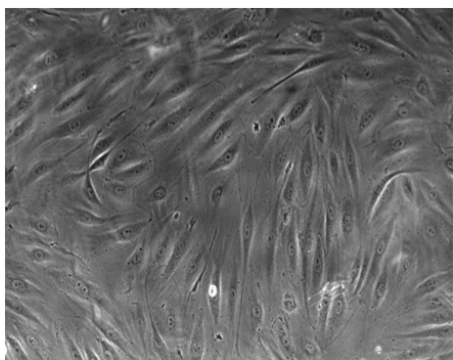
Step 5



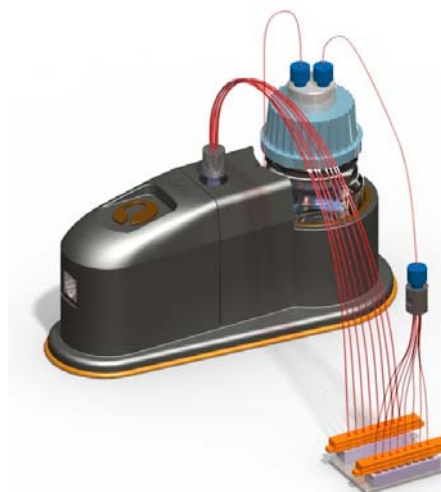
Connect tubing from media bottle to the inlet port of the pump. Connect tubing from outlet port of the pump (8 way cable with pins) to a sterile petri dish. Wash the pump using iKima App with media for 3 minutes.

Step 6

- a) Take the biochip from the incubator and place in the biosafety hood. Before connecting to the biochip, start perfusion using iKima App – typically 2 minutes perfusion, followed by 15-20 minutes pause. When media droplets form at the pins, gently connect the 8-way cable to the biochip to avoid air bubbles.



**HUVEC primary cells in Vena8 Endothelial+ biochip:
72 hrs post-perfusion with Kima pump**



- b) Connect the outlet pins to the biochip which is connected to a discard bottle or to the same media bottle for recirculation of the same media. This is done in the biosafety hood. Once connected, transfer Kima platform to a CO₂ incubator.

Note: All tubing and bottles must be autoclaved prior to the experiment.