Protocol

Microvascular & Barrier chip

1. Prepare a fibrinogen solution in EGM2 medium. The concentration can range from 4 -12 mg/mL (1).
2. Prepare a 4 U/mL thrombin solution in EGM2 medium.
3. Collect the HUVEC cells and fibroblasts according to the routine protocol.
4. Aspirate the medium from the cell pellets and resuspend both cells types in the thrombin solution (step 2). The final concentration of each cell type should be between 10 - 12 x 10^6/mL (2–5).
5. Mix 1:1 the fibrinogen solution (step 1) and the thrombin solution which contains both type of cells (step 4).

   Note: *the final concentrations must be 2-6 mg/mL fibrinogen, 2U/mL thrombin and 5-6 x 10^6/mL of each type of cell.*
6. Immediately load the mix into the central channel (Figure 1). The channel holds 6.5 µL. **!IMPORTANT**
7. Allow to polymerise for 15 minutes at room temperature.
8. Refill lateral channels with medium. Each lateral channel holds ~35 µL of medium.
9. Incubate at 37°C.
10. Change the medium in the chip every 24h.
11. After 4-7 days check the microvascular network formation under the microscope.

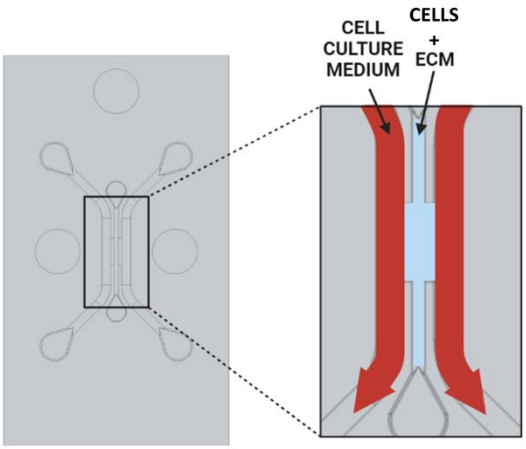

Figure 1: *schematic diagram of the cell-fibrin gel seeding. HUVEC and fibroblasts are seeded together suspended in fibrin gel and injected in the central region. Media channels are filled with EGM-2 medium.*

- General considerations

- All material used for cell cultured must be sterile. Chip plugs and tubes for the planned experiments must be autoclaved in advance.

- Avoid forming bubbles into the channels. These bubbles can affect the integrity of the adhesive cell culture.
- To avoid cell damage, the injection of any cell suspension solution should be performed slowly and allowing the re-equilibration of the internal pressure of the circuit.

- Bibliography

1. Dibble M, Di Cio' S, Luo P, Balkwill F, Gautrot JE. The impact of pericytes on the stability of microvascular networks in response to nanoparticles. Sci Rep. 7th April 2023;13(1):5729.
2. Chen MB, Whisler JA, Fröse J, Yu C, Shin Y, Kamm RD. On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics. Nat Protoc. May 2017;12(5):865-80.
3. Zhang K, Du Z, Yuan T, Huang J, Zhao X, Mi S. Long-term cultured microvascular networks on chip for tumor vascularization research and drug testing. Biomicrofluidics. 1st July 2022;16(4):044101.
4. Kim S, Lee S, Lim J, Choi H, Kang H, Jeon NL, et al. Human bone marrow-derived mesenchymal stem cells play a role as a vascular pericyte in the reconstruction of human BBB on the angiogenesis microfluidic chip. Biomaterials. 2021;279:121210.
5. Bai J, Haase K, Roberts JJ, Hoffmann J, Nguyen HT, Wan Z, et al. A novel 3D vascular assay for evaluating angiogenesis across porous membranes. Biomaterials. January 2021;268:120592.