GRADIENTECH

PROTOCOL

Flow-mediated behavioural analysis of Human Dermal Microvascular Endothelial Cells (HDMECs) - using CellDirector® 2D

CELLS AND REAGENTS: HDMECs
MV2 medium (Promocell)

Preparation of HDMECs

1. HDMECs are trypsinized and resuspended in MV2 medium (Promocell) to a concentration of 4 x 10^6 cells/ml.

Cell seeding of HDMECs to CellDirector® 2D

2. Seed 200 µl of the cell suspension into a micro chamber (CellDirector 2D, Gradientech) by pipetting slowly.

Cell culturing in CellDirector® 2D

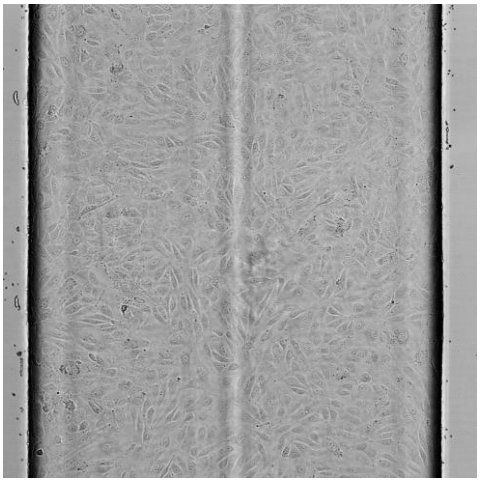
3. Culture the cells at 37°C and 5% CO_2 without flow for 3 hours until a confluent cell layer is formed in the chamber.

Place the CellDirector® 2D under the microscope

4. Place the chamber on the microscope stage (Leica SP8, humidified, 37°C and 5% CO2) and connect the inlets to 50ml syringes with culture medium, the outlet to a beaker to collect medium. Make sure all tubings are very tightly connected, no liquid leaking on the microscope and no big air bubbles go into the chamber.

Start the flow-mediated experiment and collect images

5. Pump the culture medium (MV2, pre-warmed at 37°C) through the flow chamber at a speed of 150µl/min (7.5 dyne/cm^2). Acquire bright field images every 5 minutes for 5 hours.
   Cell movement is analysed using MTrackJ (in Fiji).



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