PROTOCOL

Protocol for chemotaxis of human peripheral blood B-cell-derived cell lines MAVER-1 (mantle cell lymphoma origin) and MEC-1 (chronic lymphocytic leukemia origin)

CELLS AND REAGENTS: MEC-1 and MAVER-1 cell lines
RPMI1640 medium containing 10 % FBS
Fibronectin
CCL19

Note: *All media should be equilibrated before use (overnight at 37 °C in an incubator) to avoid bubble formation during the experiment. This applies to the RPMI1640 medium, Milli-Q water for fibronectin dilution and PBS for cell washes.*

Preparation of media

1. Equilibrate RPMI1640 medium containing 10 % FBS overnight at 37 °C in an incubator.
2. The next day, mix part of the medium with the desired chemoattractant (e.g. 200 ng/mL recombinant chemokine CCL19, e.g. 361-MI R&D Systems). To visualize the established gradient, the media can be mixed with a fluorescent marker in 1:20 ratio (e.g. TRITC 20K, 81-001, Gradientech).
3. Fill syringe 1 with control medium (no chemoattractant) and syringe 2 with medium containing the chemoattractant (and fluorescent marker). Keep at 37 °C in the incubator until use.

Pre-coating of the CellDirector 2D surface with fibronectin

Note: *Both MEC-1 and MAVER-1 cells are cell lines cultured in cell suspension. MEC-1 cell line tends to adhere weakly to the plate surface, however MAVER-1 do not adhere at all. Therefore, surface coating is crucial for these experiments.*

1. Dilute the human plasma-derived fibronectin (e.g. 1918-FN-02M, R&D, stock 1 mg/mL) to a final concentration of 50 µg/mL in Milli-Q water (1:20).
2. Inject 200 µL of the solution to the CellDirector 2D with a pipette. Coat overnight at 37 °C in incubator. Keep the device in a 10 cm petri dish containing a wetted tissue to avoid evaporation of the solution.
3. Next day, replace the fibronectin solution with equilibrated medium - wash twice with 200 µL of the media and then let block the surface for 30 min at 37 °C in the incubator.

Preparation of cells

Note: *To simplify the cell-tracking during experiments, the cells can be stained directly before use by membrane-permeable dyes (e.g. Calceins)*

1. Harvest the cell suspension from the cell culture flask, spin down (200 x g, 5 min) and wash twice with PBS.
2. *Optional washing step by Calcein Green, AM (C34852, Thermo Fisher Scientific): 10 min of incubation in 2 µM Calcein Green solution diluted in PBS, followed by 1-2 washing steps. Optimal concentration of the cell suspension: 1-2 x 10^6 cells/mL.*
3. Resuspend the cells in the equilibrated medium at a final concentration of 1-2 x 10^6/mL.

Loading the cells

1. Inject slowly the 200 µL of the cell suspension to the CellDirector.
2. Incubate for 1 hour in the incubator, in a 10 cm Petri dish. Verify proper cell adhesion by microscope.
3. Set the syringes to the pump and place the CellDirector to a holder on your microscope. Carefully attach the tubes with media and mark the tube containing chemoattractant. Attach the tube with vacuum to the device.
4. Set the CO_2 chamber in case of longer experiments.
5. Start the experiment. The injection speed should be optimized. For these cells, initial speed 5 µL/min was used to set the gradient and then was decreased to 1 µL/min. In case of low cell adherence, the loose cells can be removed from the device by a transient faster medium flow.
6. Collect images using bright field (or fluorescence) microscopy (10x objective) at 0.5 fpm.

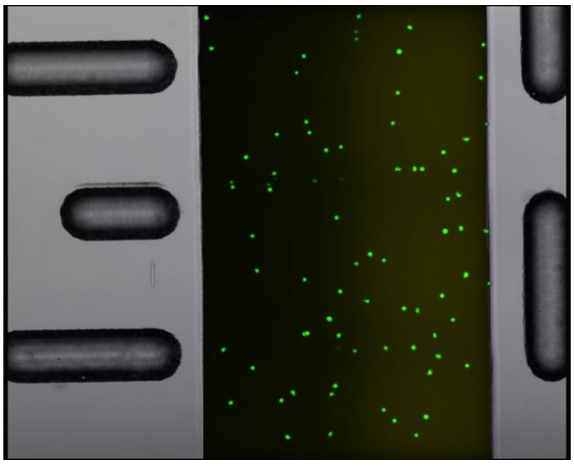

Fig. 1: *Calcein-labelled B cell lymphoma-derived cells in CellDirector® 2D gradient channel, with gradient marker.*

Protocol modification – switch of treatments (CellDirector® Switch, 71-001, Gradientech)

Note: *To change the experimental conditions throughout the running experiment, a CellDirector Switch connected to an additional syringe (e.g. containing inhibitor of the chemotaxis) can be used. Do not forget to assemble this device ahead together with the remaining syringes.*

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