GRADIENTECH

PROTOCOL
Fibroblast chemotaxis towards a gradient of PDGF-B using CellDirector® 2D

CELLS AND REAGENTS NEEDED:

Fibroblasts (HFL-1)	FBS (Fetal Bovine Serum)
Trypsin	PDGF-B (Platelet Derived Growth Factor)
MEM (Minimum Essential Medium)	PBS (Phosphate Buffered Saline)

Preparation of fibroblasts

1. Culture fibroblasts in MEM medium supplemented with 10% FBS at 37°C in a humid atmosphere with 5% CO_2.
2. Trypsinise the fibroblasts (5-10 min), than deactivate the trypsin with MEM medium supplemented with 10% FBS.
3. Count and transfer 1x10^6 fibroblasts to a new tube.
4. Re-suspend the 1x10^6 fibroblasts in 200 µL MEM medium supplemented with 10% FBS.

Load fibroblasts into CellDirector® 2D and induce chemotaxis towards PDGF-B

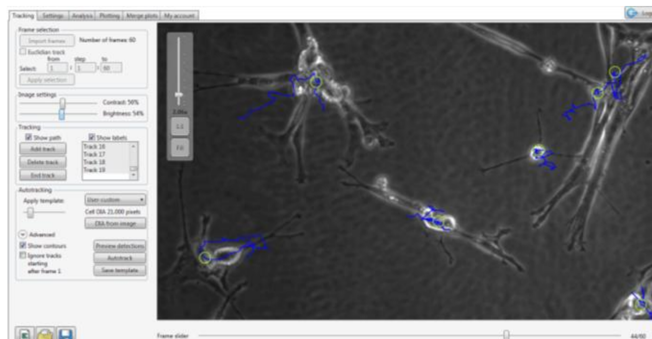
1. Pipet 200 µl fibroblast suspension into an empty CellDirector® 2D.
2. Let the fibroblasts adhere (2-3 hours) by placing the CellDirector® 2D assay, with the green side facing up, in a humidified incubator.

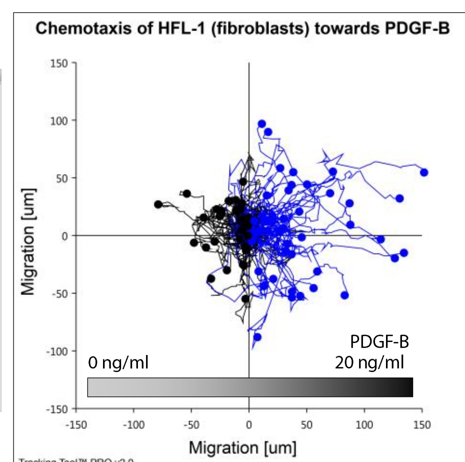
   NOTE: Typically, about 20% of the fibroblasts will adhere after 3 hours of incubation, which is ideal for migration experiments. If no fibroblasts have adhered after 3 hours, they will probably not adhere at all. Wash the CellDirector® 2D assay with PBS and load new fibroblasts.

3. Change to starvation medium by slowly pipetting 200µL MEM medium (no FBS) into the assay.
4. After 3-4 hours of starvation, prepare the syringes by filling one syringe with MEM medium and the second syringe with PDGF-B [20 ng/ml] in MEM medium.
5. Start the CellDirector® 2D experiment as described in the *Short User Guide*. See also the *Guide for Flow Directions and Gradient Orientation*.
6. Collect time-lapse images using bright-field microscopy (10x objective) at 0.2 fpm (1 image every 5 min). Preferably, collect images at serial ROI along the gradient channel, as well as from the two control channels.
7. Continue collecting images for at least 4 hours (50 cycles).
8. Analyse using Tracking Tool™ PRO software (www.gradientech.se).

RESULTS:

Total number of tracked cells	77
Cells migrating towards increasing PDGF-B	60%
Average velocity [µm/min]	0.6
Directionality	0.27





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