APPLICATION NOTE

GRADIENTECH

Chemotaxis of human neutrophils towards a stable gradient of IL-8

Chemotaxis is the process of directed cell migration in gradients of molecular substances, chemoattractants. CellDirector® 2D is an easy-to-use assay, that generates a stable gradient and maintains it throughout the entire experiment. CellDirector® assays give the possibility to record the migration paths of individual cells within stable gradients as well as control regions, generating results of the highest quality.

Here, we demonstrate how a single experiment with CellDirector® 2D produces results that confirm that interleukin-8 (IL-8) potently induces chemotaxis of human neutrophils.

Overview of experimental steps	Estimated time per step
1. Neutrophil isolation from human blood	2 h
2. Neutrophil activation	5 min
3. Neutrophil seeding into CellDirector 2D	1 h
4. Starting the experiment	10 min
5. Data collection by time-lapse microscopy	2 h
6. Data analysis	1 h

Benefits of CellDirector® 2D

CellDirector 2D is a cell-based assay for high-quality cell migration analysis. This assay forms **controlled and well-established gradients** of your active substance as soon as the experiment is initiated. The chemotactic cell response analysis is based on **cell tracking** where the migration paths of the individual cells can be visualised. **Excellent cell culture conditions** together with the possibility to **distinguish between chemotaxis and random cell migration** bridges the gap between *in vitro* and *in vivo* conditions.

CellDirector® 2D overview

Two different cell culture media solutions are used for each CellDirector 2D experiment. The source medium contains the substance to be evaluated. For most experiments, the sink medium contains only serum-free cell media, but shallower gradients can be acheived by using lower concentrations of the active substance in the sink medium.

Gradients are formed in the centrally positioned **gradient region** by diffusion of the chemotactic substance between fluid streams generated from the two different media solutions. Importantly, CellDirector 2D also features **two control regions** where cells experience no gradient, but instead are exposed only to the source or sink media solution.

Neutrophil isolation from human blood

In the present example, neutrophils were initially isolated from human peripheral blood. Whole blood was withdrawn from a healthy donor and neutrophils isolated according to a well-established protocol using dextran sedimentation and Ficoll-Paque (GE Healthcare) gradient centrifugation.

Neutrophil activation and seeding

The neutrophils were activated by incubating the cells for 5 minutes with 50 ng/ml of IL-8. The cell suspension was centrifuged, washed and diluted to a final concentration of 0.25 x10^6 cells/ml.

200 µl of the neutrophil suspension was seeded directly into CellDirector 2D via the single outlet tube. No matrix coating was needed as the neutrophils adhered readily to the glass substrate of the assay.

CellDirector 2D was incubated (green side up) at 37 °C, 5% CO_2 for 1 h to allow cells to adhere.

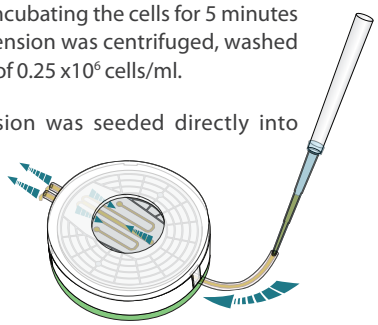
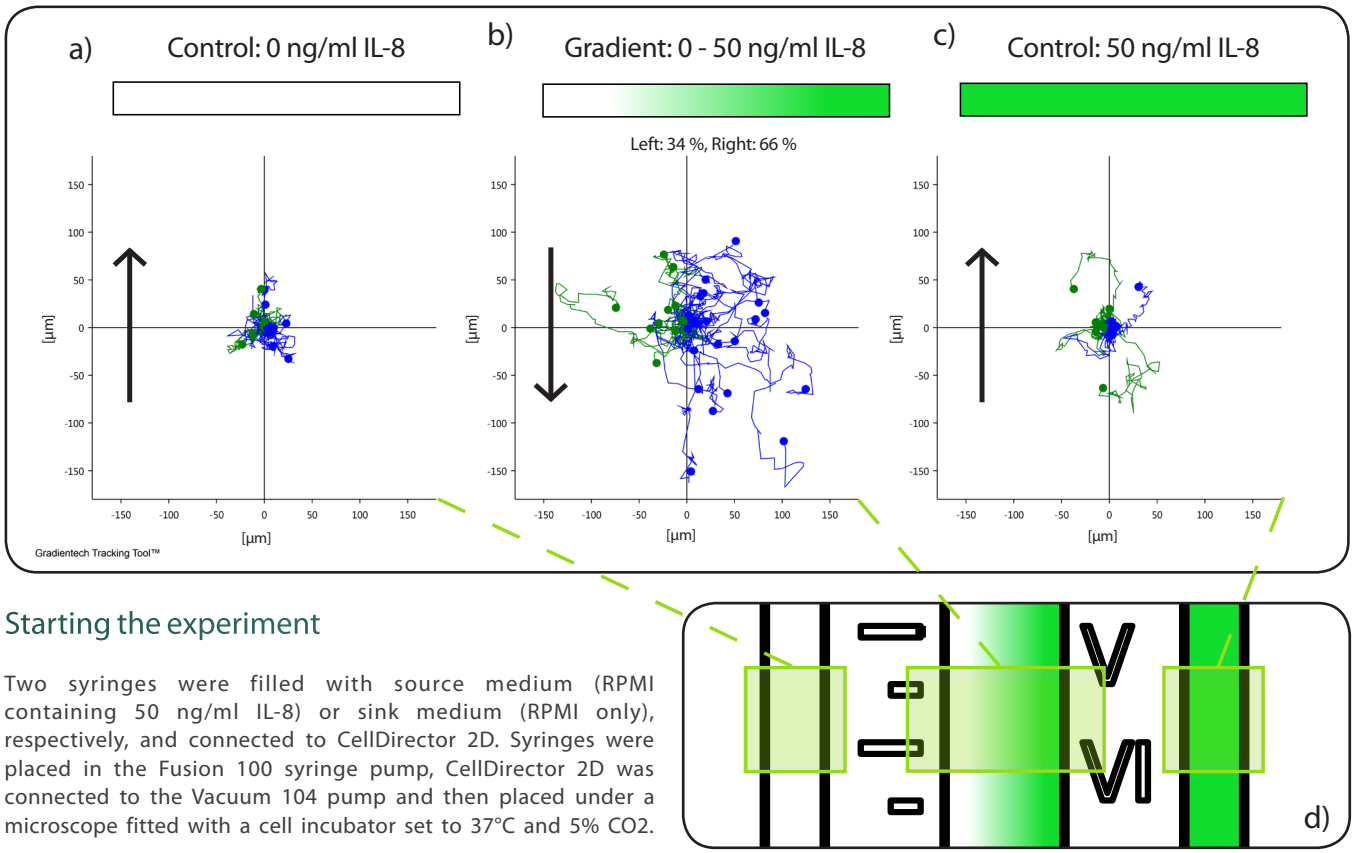


Figure 1. Loading of neutrophils into CellDirector 2D is easily achieved by manual pipetting into the single outlet tube.



Starting the experiment

Two syringes were filled with source medium (RPMI containing 50 ng/ml IL-8) or sink medium (RPMI only), respectively, and connected to CellDirector 2D. Syringes were placed in the Fusion 100 syringe pump, CellDirector 2D was connected to the Vacuum 104 pump and then placed under a microscope fitted with a cell incubator set to 37°C and 5% CO2.

Data collection by time-lapse microscopy

Time-lapse imaging was used to collect images for 2 hours from the chemotaxis region as well as from the two internal control regions. Brightfield images were collected every minute due to high neutrophil migration velocities.

Data analysis

Cells in the chemotaxis region and the control regions were tracked using the freely available Gradientech Tracking Tool™ software. The plots clearly show that a gradient of IL-8 ranging from 0-50 ng/ml very potently induces chemotaxis of a subset of human peripheral blood neutrophils towards higher levels of IL-8 (Fig. 2).

Figure 2. Migration plots of neutrophils exposed to a) 0 ng/ml IL-8 (control), b) a gradient of 0-50 ng/ml IL-8 and c) 50 ng/ml IL-8 (control). Black arrows show the direction of fluid flow. In the middle chemotaxis region of panel b), blue tracks indicate cells that have migrated towards increasing levels of IL-8 (66 % of all tracked cells). Green tracks indicate cells that have migrated away from increasing levels of IL-8 (34 % of all tracked cells). d) Channel positions where images were collected.

Product information

PRODUCT	SUITABLE CELLS	APPLICATIONS	CATALOGUE #	SIZE
CellDirector® 2D	All types of adherent cells	Chemotaxis analysis of for example cancer cells, endothelial cells, neutrophils or neural cells.	REF 11-001-10	10 assays/box

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