A NEW TECHNOLOGY FOR IN VITRO CHEMOTAXIS ASSAYS

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Introduction

- Chemotaxis is the movement of a cell in a direction corresponding to a gradient of increasing or decreasing concentration.
- Chemotaxis is a fundamental element of normal and pathological cell biology.
- Traditional in vitro methods for studying cell migration include:
  - Scratch or Cell Exclusion Assays: These are not measures of directed cell migration or chemotaxis. For the most part, they are a measure of "random" migration.
  - Microfluidic Chemotaxis Assays: Researchers can see the cells, but they suffer from small gradients across the cell, low participation rates, and low throughput.
- Traditional Boyden Chamber Assays: This predominant industrial approach has good throughput (96-wells). However, the researcher can easily visualize the process of cell migration, it requires many cells, and additional labeling or manual cell counting.

This poster describes a novel approach that combines hardware, software algorithms, and a consumable to provide a fully automated, integrated solution for studying chemotaxis using live-cell imaging.

Incucyte ClearView Cell Migration Plate

- Visualize Chemotaxis – The ClearView Plate incorporates an optically smooth membrane surface enabling acquisition of high-definition, phase-contrast images. Standard Boyden Chamber surfaces are not easily amenable to imaging.
- Persistent Gradient – The low porosity of the ClearView Plate results in a gradient that is stable for over 72 hours compared to 4-6 hours in traditional consumables.
- Low Cell Density – The combination of a long-term, persistent gradient and the interest in visualizing chemotaxis has resulted in an assay that requires significantly fewer cells compared to traditional Boyden Chamber Assays.
- Integrin Signaling – In the ClearView Plate, cells are required to migrate to the pores. This requires integrin interactions with the substrate that likely are not required in traditional Boyden Chamber consumables.
- Automated Image Processing – The unique design of the ClearView Plate facilitates quantitation of cells on top and the bottom of the membrane.

Incucyte ZOOM®

- Automated, Label Free Quantitation
- T cell Chemotaxis Towards CXCL11

The phase-contrast image is blended with image segmentation mask (yellow) created by an automated image processing algorithm.

- Optically-clear surface for label-free imaging within Incucyte ZOOM®
- Cells are added to the upper chamber and chemotactant to lower reservoir plate.
- Chemotactic cell migration towards the pores is automatically analyzed using the Incucyte ZOOM® instrument.

T Cell Chemotaxis to SDF-1α and CXCL11

- 5,000 CD3/CD28 Dynabead-activated T cells were seeded in each well on an ICAM-1 coated surface. The indicated chemotacticant was added to the reservoir plate. Data were collected over a 36 hour period at 1hr intervals. A and C: Kinetic curve of concentration-dependent responses to SDF-1α and CXCL11. Data represent mean ± SEM. N=6. B and D: SDF-1α and CXCL11 agonist at 10 µM. Data represent the mean ± SEM. N=6 per condition. E: Each well is individually graphed in a microplate graph overview, illustrating well-to-well reproducibility. F: A representative image acquired at first time point of the assay from control wells (no chemotacticant). Lamellipodia/Filopodia on T cells indicates that the cell are actively interacting with the surface.

Neutrophil Chemotaxis to IL-8, fMLP, and C5a

- 5,000 neutrophils per well were seeded on a Matrigel + PBS coated surface. The indicated chemotacticant was added to the reservoir plate. Data were collected over a 6 hour period at 30 min intervals. A and C: Kinetic curve of concentration-dependent responses to IL-8 and fMLP. Data represent mean ± SEM. N=6. B and D: IL-8 and fMLP against curve at 4 h. Data represent mean ± SEM. N=4 per condition. E: Each well is individually graphed in a microplate graph overview, illustrating well-to-well reproducibility. F: A representative image acquired at first time point of the assay from control wells (no chemotacticant). Blurry cells are located on the bottom side of the membrane.

Specific inhibition of T cell chemotaxis using CXCR3 and CXCR4 inhibitors

- Kinetic inhibition and pharmacology of AMD3100 and NBI 74330. CD3/CD28 Dynabead-activated T cells were plated at a density of 5x10⁶/well on a coated ClearView insert (Protein G + ICAM). AMD 3100 [A and B] or NBI 74330 [D and E] was added to the reservoir plate at 100 nM or 50 nM, respectively. Each data point represents mean ± SEM. N=6. AMD3100 inhibitor has a clear selective effect on CXCR4-mediated chemotaxis towards SDF-1α (IC50 AMD 3100 = 197 nM). No effect of AMD 3100 was found in CXCR3-mediated chemotaxis towards CXCL11. NBI 74330 has a clear effect on CXCL11-mediated chemotaxis (IC50 NBI 74330 = 17.8 nM). NBI 74330 weakly inhibited chemotaxis towards SDF-1α.

Summary and Impact

- Real-time visualization and automated analysis of chemotactic cell migration in a 96-well format within your incubator
- Measure label-free, or labelled cell migration with fixing, staining or cell scraping steps
- Setup and walk away – fully automated image based analysis
- Highly reproducible 96-well approach suitable for profiling and screening
- Investigate cell migration on biologically relevant surfaces
- Sustained and stable gradient over 72 hours