BioResearch

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True Chemotaxis Discovery by Real-time Imaging of Cells in Stable Chemoattractant Gradient Using CellDirector® 2D and the CytoSMART™ System

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Introduction

Directed migration of cells towards molecular gradients, i.e. chemotaxis, plays an essential role in many biological processes, such as inflammation, cancer metastasis and wound healing. *In vitro* transwell assays have long been the most common method for studying cell migration. However, the molecular gradients are undefined and the support for live cell microscopy is weak, limiting the information obtained by this method. Furthermore, the transwell method does not reveal the cells' migration directions or velocities, and as a result, this method cannot differentiate between chemokinesis, i.e. increased random movement, and chemotaxis.[1] New methods are therefore needed for dynamic and precise quantification of cell migration in response to stimuli.

Microfluidic technology combined with live cell imaging and cell tracking can overcome these limitations by monitoring and quantifying cell responses over time, while simultaneously creating defined and precise chemoattractant concentration gradients.

CellDirector® 2D is a microfluidic assay optimized to study cellular migration to stimuli. A continuous flow of cell media, as well as cell media containing chemoattractant, exposes the cells to a stable gradient throughout the entire experiment.[2] By following cell responses in real-time, true chemotaxis can be distinguished from random cell migration. The cell migration experiment is performed inside an incubator and the cell responses are followed over time by real-time imaging using the CytoSMART™ System. The CytoSMART™ System consists of a mini-microscope, i.e. the CytoSMART™ Device, accompanied with a tablet utilizing a cloud-based system for real-time access to cell cultures. Due to its large imaging area, cell responses from both the gradient channel and a control channel of CellDirector® 2D can be collected simultaneously. Images are analyzed using the cell tracking software Tracking Tool™ PRO.

Fibroblasts isolated from patients with inflammatory lung diseases have abnormal properties compared to fibroblasts isolated from normal lungs. Chemotactic properties of fibroblasts in the pathogenesis of pulmonary fibrotic diseases may be an important aspect of disease progression. Previous research indicates an increased chemokinetic response of fibroblasts obtained from fibrotic lungs towards platelet-derived growth factor (PDGF), but the question if chemotaxis is enhanced remains.[3] In this study, we compared the responses of normal human lung fibroblasts (NHLF) with diseased human lung fibroblasts (DHLF) isolated from adult lung tissue of asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD) patients. The chemotactic responses of the different cell types to a gradient of PDGF beta subunit homodimers (PDGF-BB) were quantified using the CellDirector® 2D Assay in combination with the CytoSMART™ System.

General Materials

- CytoSMART™ Lux 10X System, Lonza
- CellDirector® 2D Microfluidic Cell Migration Assay, Gradientech
- CellDirector® Holder, Gradientech
- Tracking Tool™ PRO Cell Tracking Software, Gradientech

Methods

Cell Culture

NHLF (CC-2512; Lonza) and DHLF including fibroblasts isolated from adult lung tissue of asthma (194912; Lonza), COPD (195277; Lonza) and cystic fibrosis (194843; Lonza) patients were cultured in FGM™-2 Growth Medium (CC-3132; Lonza). Cells, at passage 4 – 8, were seeded and grown until 80% confluence. Cells were washed with HEPES buffer

and trypsinized with Trypsin/EDTA (CC-5012; Lonza) for 5 – 10 minutes. The trypsinization process was deactivated using Trypsin Neutralizing Solution (CC-5002; Lonza). The cell suspension was centrifuged and the pellet re-suspended in 1 ml FGM™-2 Medium. Cells were counted and the cell density was adjusted to a concentration of 0.75 – 1 × 10^6 cells/ml.

CellDirector® 2D Migration Assay

The migration experiments were performed in triplicates for all diseased fibroblast cell types according to the following protocol:

1. **Day 1:** Pipet 200 µl of the cell suspension into an empty CellDirector® 2D (11-001, Gradientech).
2. Let cells adhere by incubating the CellDirector® 2D overnight in a sterile petri dish containing a wet tissue. Incubate all cell media overnight in Falcon tubes with lids slightly open for degassing purposes.
3. **Day 2:** Prepare the syringes by filling one syringe with serum-free FGM™-2 Medium and the second syringe with PDGF-BB (25 ng/ml) in serum-free FGMT™-2 Medium. Connect the associated tubes to the syringes.
4. Place the syringes in a syringe pump. Start pump at 1 µl/min.
5. Connect both tubes to the inlet connectors of CellDirector® 2D.
6. Fit CellDirector® 2D in the microscopic CellDirector® Holder (70-001, Gradientech) for stable imaging, and place the holder under the CytoSMART™ Device.

Image Acquisition and Analysis

Fibroblast migration responses were monitored in real-time using the CytoSMART™ Lux 10X System. Images were collected at regular intervals of 7.5 minutes (10x magnification) for 5 hours. Images were transmitted to the cloud and downloaded after completed experiment (.jpg format). In order to quantify chemotaxis, individual cell movement was analyzed for each data set using Tracking Tool™ PRO v2.0 (31-001, Gradientech). Cells were tracked manually in the presence and absence of a chemoattractant gradient and important migration parameters were computed automatically by the software. The trajectory of cells was plotted on a polar coordinate grid.

Figure 1
Setting up a cell migration experiment with the microfluidic CellDirector® 2D Assay and the CytoSMART™ System.

Results

Time Lapse Imaging

During the experiment, the fibroblast migration responses were monitored in real-time for 5 hours, resulting in image series of 40 images. As exemplified in Figure 2, the CytoSMART™ System captures the entire 1 mm wide gradient channel (left in image) and an entire 0.5 mm wide control channel (right in image) in the CellDirector® 2D Assay. The imaging area of the CytoSMART™ System measures 2.5 mm × 1.7 mm.

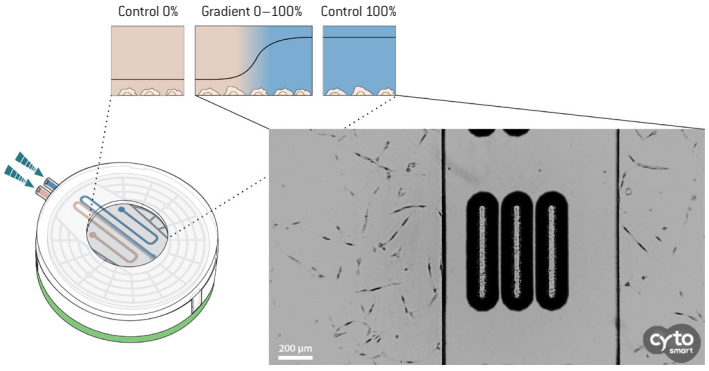


Figure 2
Example of a captured image during a cell migration experiment using CellDirector® 2D. The diseased asthma fibroblasts in the left gradient channel are exposed to a stable gradient ranging between 0 – 25 ng/ml PDGF-BB across the 1 mm wide channel, whereas the fibroblasts in the positive control channel are exposed to 25 ng/ml PDGF-BB only.

Quantification of Chemotaxis

After the completed migration experiment, the image sequence was analyzed and cells were tracked individually using the Tracking Tool™ PRO Software. The gradient channel and the control channels were treated separately and migration data between the experiments were compared. The software generates statistical results for parameters that quantify migration, e.g. the center of mass displacement. Center of mass (COM) is the mean total displacement of all tracked cells, and is an important indicator of chemotaxis when comparing migration within the gradient channel (+/-) and the control channels (positive +/+; negative -/-). The displacement of the COM was calculated in the direction of chemoattractant (0 – 25 ng/ml PDGF-BB) for the gradient channel, as well as for the positive (25 ng/ml PDGF-BB) and negative control (0 ng/ml PDGF-BB) channels for all fibroblast cell types. As seen in Figure 3, the chemotactic responses are most apparent for fibroblasts derived from asthma and cystic fibrosis patients. COPD and non-diseased fibroblasts respond with chemotaxis towards PDGF-BB, however to a lower degree compared to asthma and cystic fibrosis fibroblasts.

The Tracking Tool™ PRO Software also presents cell trajectory plots, so called polar plots, where each track represents the migration path of an individual cell with its starting point in the center of the plot (0,0). Figure 4 shows, as an example, the merged migration plot for triplicate experiments of asthma fibroblasts migrating along a stable PDGF-BB gradient. The tracks depicted in blue represent cells that have migrated towards increasing PDGF-BB concentrations. As obvious by the polar plot and the migration statistics, fibroblasts isolated from lungs of asthma patients display chemotaxis towards increasing concentrations of PDGF-BB.

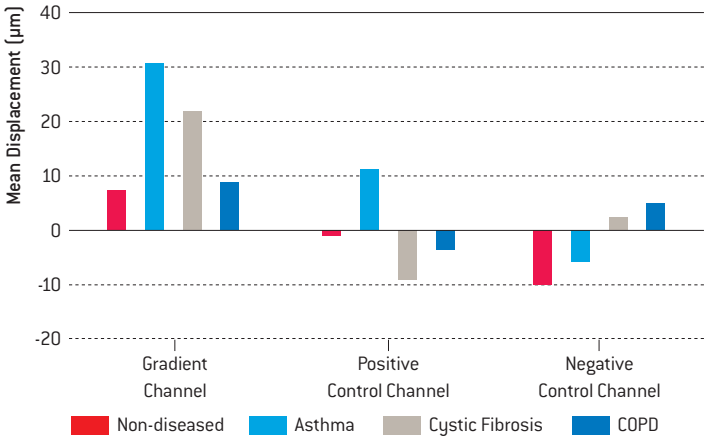


Figure 3

Center of mass (COM) displacement of the different fibroblast types as an average of all performed migration experiments. The first set of bars represents the gradient channel (0 – 25 ng/ml PDGF-BB), the second set of bars represents the positive control channel (25 ng/ml PDGF-BB), and the third set of bars represents the negative control channel (0 ng/ml PDGF-BB). The mean displacement is calculated as a weighted average based on the total amount of cells tracked for each cell type and in each channel.

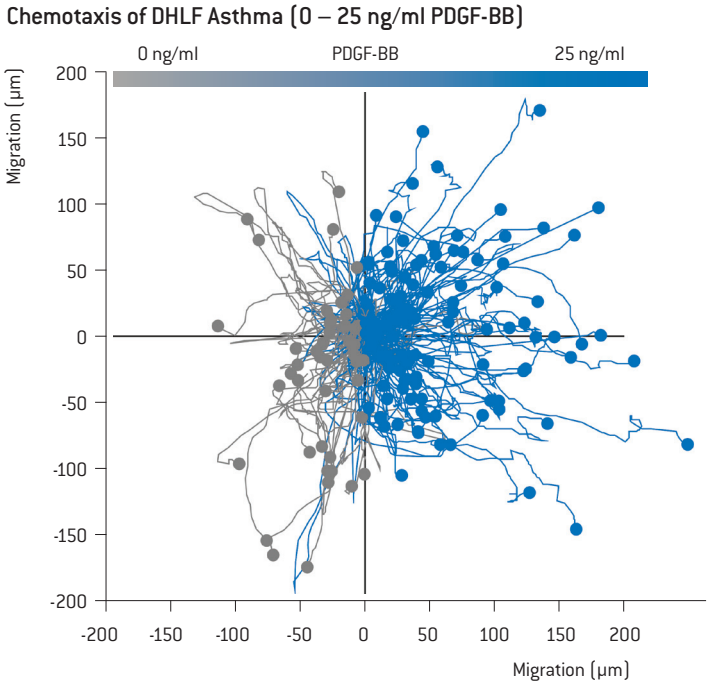
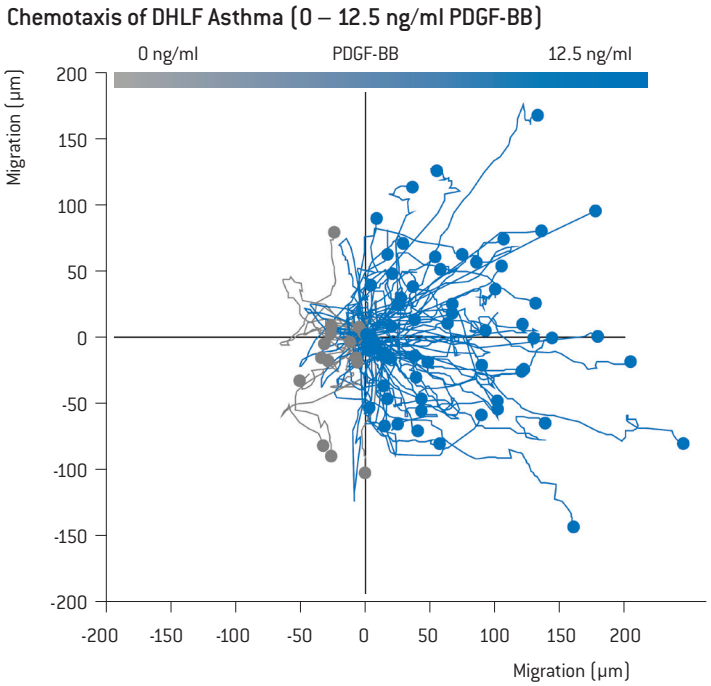


Figure 4

Polar plot of asthma fibroblasts migrating along a stable gradient of PDGF-BB (merge of triplicate experiments).



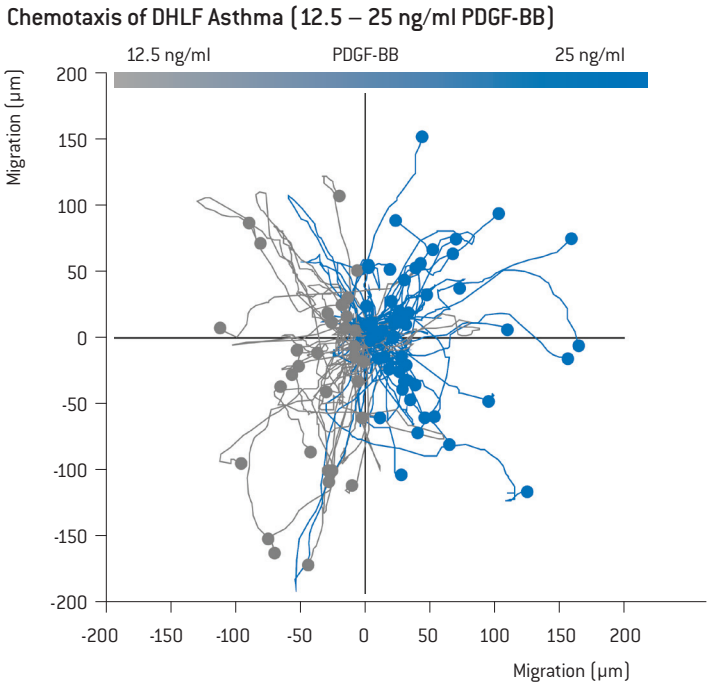


Figure 5 (top and above)

Polar plots indicating a dose response relationship for asthma fibroblasts and the PDGF-BB chemoattractant.

Dose Response Relationship

The Tracking Tool™ PRO Software offers the possibility to plot and analyze cells in specific regions in the captured images. We used this feature to discover any differences in chemotactic responses depending on the PDGF-BB concentration profile. This is a way to discover a dose response relationship of cells and the chemoattractant. As seen in Figure 5, the diseased asthma fibroblasts respond with significant chemotaxis towards PDGF-BB in the lower concentration interval (0 – 12.5 ng/ml) when compared to the higher concentration range (12.5 – 25 ng/ml). This can be concluded from both the polar plots and by comparing their differences in velocity, COM, forward migration index and directness (Figure 6). For explanations of the statistical parameters, see the caption in Table 1. The higher concentration of PDGF-BB resulting in decreased COM displacement responses could indicate a high-dose chemotaxis arrest of asthma fibroblasts.[1] In this study, this tendency is predominantly seen for the asthma fibroblasts and not for the other diseased and normal lung fibroblasts (data not shown).

	Asthma DHLF 0 – 12.5 ng/ml PDGF-BB					Asthma DHLF 12.5 – 25 ng/ml PDGF-BB			
	Velocity (µm/min)	Center of Mass (X) (µm)	Forward Migration Index (X)	Directness		Velocity (µm/min)	Center of Mass (X) (µm)	Forward Migration Index (X)	Directness
Mean (n=3)	0.60	47	0.12	0.48	Mean (n=3)	0.53	10	0.05	0.44

Table 1

Statistical parameters for the chemotaxis responses of asthma fibroblast in the lower (0 – 12.5 ng/ml) and higher (12.5 – 25 ng/ml) PDGF-BB concentration range. Explanation of the statistical parameters; Velocity: mean migration velocity of all tracked cells; Center of mass (X): mean total displacement of all tracked cells along the chemoattractant gradient; Forward migration index (X): directness of all tracked cells (see below) along the chemoattractant gradient; Directness: the directness of each cell is the distance between the cells' start- and end-position divided by its accumulated migration distance.

Conclusion

The CytoSMART™ Lux 10X System in combination with the CellDirector® 2D Microfluidic Assay constitute a cost-effective and easy-to-use solution for performing high-quality cell migration experiments of physiological relevance. By monitoring the cells in-real time in conditions of stable and controllable chemoattractant gradients, true chemotaxis can be distinguished from random chemokinesis. Chemotaxis can be quantified by statistical parameters, such as COM displacement, migration velocity and directionality. By comparing migration velocities of cells exposed to a gradient of a chemoattractant, with the velocities of cells in the positive and negative control channels, chemotaxis can easily be distinguished from pure chemokinetic responses.

In this study, we show that fibroblasts isolated from the lungs of patients with asthma and cystic fibrosis respond with chemotaxis to a larger degree, compared to those fibroblasts isolated from COPD patients and normal lung fibroblasts. Among the tested fibroblast cell lineages, the migration analysis additionally indicates a dose-dependent chemotactic response specific for asthma fibroblasts. However, we want to emphasize that the migration experiments were based on a single donor for each specific disease and non-diseased condition and more data is needed to conclude these findings.

To summarize, the large field of view of the CytoSMART™ System allows for real-time imaging of gradient and control conditions within the CellDirector® 2D Assay, constituting a markedly less expensive solution compared to regular time-lapse microscopes with movable stages.

References

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