Chemotaxis of HUVEC in 2D and 3D

1. General Information

This is a detailed protocol for analyzing the chemotaxis of HUVEC (human umbilical vein endothelial cells) using the µ-Slide Chemotaxis^{3D}. This slide can be used for two-dimensional (2D) as well as for three-dimensional (3D) experiments. Example data for cells cultured on a 2D surface, in bovine or rat tail collagen type I gels or in Matrigel® are given in this Application Note. A 10% FCS gradient was used to stimulate the directed migration of HUVEC. More general information about handling the slide, experimental planning and troubleshooting is provided in Application Note 17 "*3D Chemotaxis Assays using µ-Slide Chemotaxis* ^{3D} ".

2. Equipment and Material needed

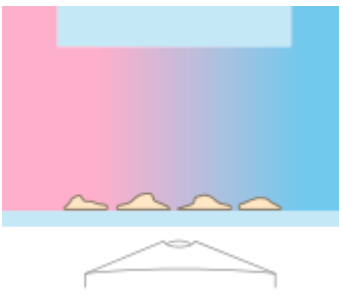
A live cell imaging set-up is a prerequisite for performing migration studies using the µ-Slide Chemotaxis^{3D}. A motorized stage is recommended to be able to observe all 3 chambers of one slide or even several slides at the same time. Detailed information about the hardware and the software needed can be found in Application Note 17.
The 2D as well as the 3D experiments were performed using the µ-Slide Chemotaxis^{3D} (ibidi, 80322). The 3D environment was provided by embedding HUVEC in either collagen type I gels or Matrigel®. A list of all needed ingredients for the collagen gels and detailed protocols can be found in Application Note 26 "Collagen I gel for 3D Cell Culture". The composition of a 30% Matrigel® solution is given in table 2. For general Matrigel® handling information, please read the manufacturer information.

3. Procedure

The µ-Slide Chemotaxis^{3D} was used for both 2D and 3D experiments. Detailed illustrated information about handling this slide, experimental planning and troubleshooting can be found in Application Note 17.

3.1. Performing 2D Chemotaxis Experiments

- Cell type: HUVEC
- Cell culture surface: Collagen IV
- Final cell concentration: 3 x 10^6 cells/ml
- Attractant: 10% FCS
- Time lapse measurement: 24 h every 10 min
- Objective: 4x (phase contrast)



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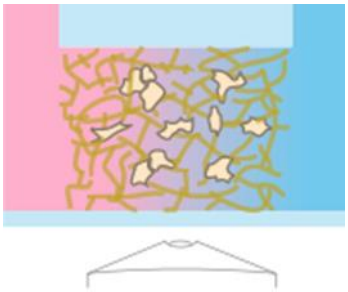
Prepare the cell suspension as usual and dilute it to the desired cell concentration. Pipette the cell suspension into the channel and let your cells attach to the surface. Start the time lapse measurement after filling medium and attractant in the reservoirs.

3.2. Performing 3D Chemotaxis Experiments



- Cell type: HUVEC
- Cell culture surface: ibiTreat
- Final cell concentration: 2 x 10^6 cells/ml
- Attractant: 10% FCS
- Collagen Type I Gels: bovine and rat tail collagen gels
- Matrigel®
- Time lapse measurement: 24 h every 10 min
- Objective: 4x (phase contrast)

Prepare the cell suspension as usual and dilute it to the desired cell concentration. Mix the cell suspension and the collagen gel mix/Matrigel® thoroughly and apply the mixture into the channel. After gelation and filling of the reservoirs, place your slide on the microscope and start the time lapse measurement.

Table 1 Composition of a 1.5 mg/ml bovine and rat tail collagen type I gel. All ingredients are listed in order of pipetting. See Application Note 26 for the full protocol.

Ingredient	bovine (RT)	rat tail (one ice)
10x M199	20 µl	20 µl
H_2O	20 µl	79 µl
$NaHCO_3$ 7.5%	10 µl	11 µl
1x ECBM (without supplements and FCS)	50 µl	50 µl
Collagen I	150 µl	90 µl
Cell suspension	50 µl	50 µl
Total	300 µl	300 µl

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Table 2 Composition of a 30% Matrigel® solution. All ingredients are listed in the order of pipetting.

Ingredient	Matrigel® solution (on ice)
1x ECBM (without supplements and FCS)	160 µl
Matrigel®	90 µl
Cell suspension in 1x ECGM	50 µl
Total	300 µl

3.3. Chemotaxis Experiment

A reliable experimental setting includes not only the chemotaxis experiment (+/-) itself, but also two control experiments (+/+, -/-). The latter are performed by filling the chamber completely with chemoattractant solution (+) or with chemoattractant free (-) medium. These control experiments help to determine whether a compound is influencing the directed movement of cells and/or random migration.

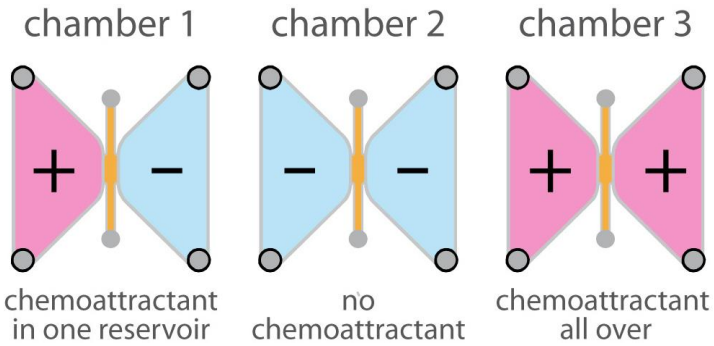

Figure 1 Recommended setup for one slide.

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4. Example: Comparison of 2D and 3D Experiments using 10% FCS as Chemoattractent

The migration behavior of cells is depended on the cell environment and on the chemoattractant being used. The example data shown here outlines the differences in migration behavior of HUVEC cultured on a 2D surface (Collagen IV) and embedded in a rat tail or bovine collagen type I gel (1.5 mg/ml) or Matrigel (30% Matrigel®). 10% FCS was used as chemoattractant for all culture conditions. Cell migration was analyzed between time point 6 hours and time point 18 hours to ensure homogeneous cell behavior, and at the same time also to prevent effects caused by nutrient deprivation at later time points. To minimize starvation effects in the negative control experiment, 0.1% BSA was added to the medium for the 2D experiments and cells were embedded in gels containing 1.7% FCS for the 3D experiments.

Directed cell migration can be assumed if the following parameters are fulfilled with statistical relevance: 1) the FMI^{II} value of the chemotaxis experiment should be larger than the $FMI^{\perp}$ and the p-value should be $p<0.05$; 2) the FMI^{II} and the $FMI^{\perp}$ of each control experiment should be around zero and the p-value should be $p>0.05$.

Table 3 Comparison of the migration parameters of HUVEC cultured on a 2D surface or embedded in either bovine or rat tail collagen type I gels or Matrigel. Cell migration was analyzed from time point 6 hours to time point 18 hours. The data of the migration parameters of the control experiments is not given here.

	2D (Collagen IV)	3D (Bovine collagen gel)	3D (Rat tail collagen gel)	3D (Matrigel®)
FMI^{II}	0.13	0.16	0.12	0.27
$FMI^{\perp}$	0.02	-0.01	-0.02	-0.05
Directness	0.23	0.29	0.24	0.42
Euclidean distance [µm]	196.78	89.28	37.64	45.9
Velocity [µm/min]	1.16	0.37	0.21	0.16
Rayleigh Test [p-value]	2.2e-04	0.006	0.02	8.2e-05

A chemotactic response could be observed for all four tested culture conditions. Both the FMI^{II} and the p-value (of the Rayleigh test) of the chemotaxis experiments indicate a directed cell migration. However, the migration parameters Euclidean distance and velocity differ significantly depending on the culture condition tested. Cells cultured on a 2D surface covered distances of up to 190 µm with an average velocity of 1.1 µm/min. Contrary, a reduction of velocity of cells embedded in bovine or rat tail collagen type I gels or Matrigel resulted in an average covered distance of

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only 90, 37 and 46 µm. The differences in velocity of HUVEC embedded in different extracellular matrixes can be explained by different fibrillar matrix architectures. Differences in collagen matrices, for example, are described in detail by Peter Friedel and colleagues in their paper “Physical limits of cell migration: Control by ECM space and nuclear deformation and tuning by proteolysis and traction force” (J. Cell Biol. Vol. 201 No. 7 1069-1084, June 24, 2013)

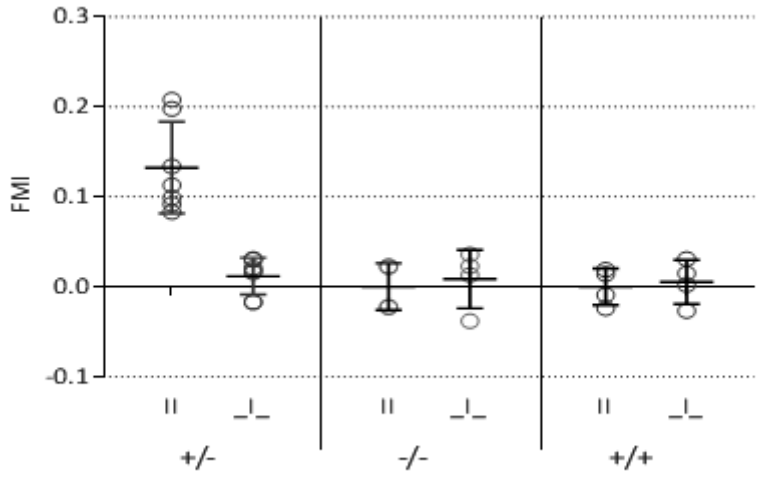

Figure 2 Comparison of the FMI values of 3D chemotaxis experiments and the corresponding controls. The data shown was obtained from HUVEC which were embedded in a bovine collagen type I gel and exposed to a FCS gradient. Directed migration can be assumed if the FMI^{II} value of the chemotaxis experiment is larger than the $FMI^{\perp}$ and if the FMI values of the control are around zero. Each dot represents one experiment.

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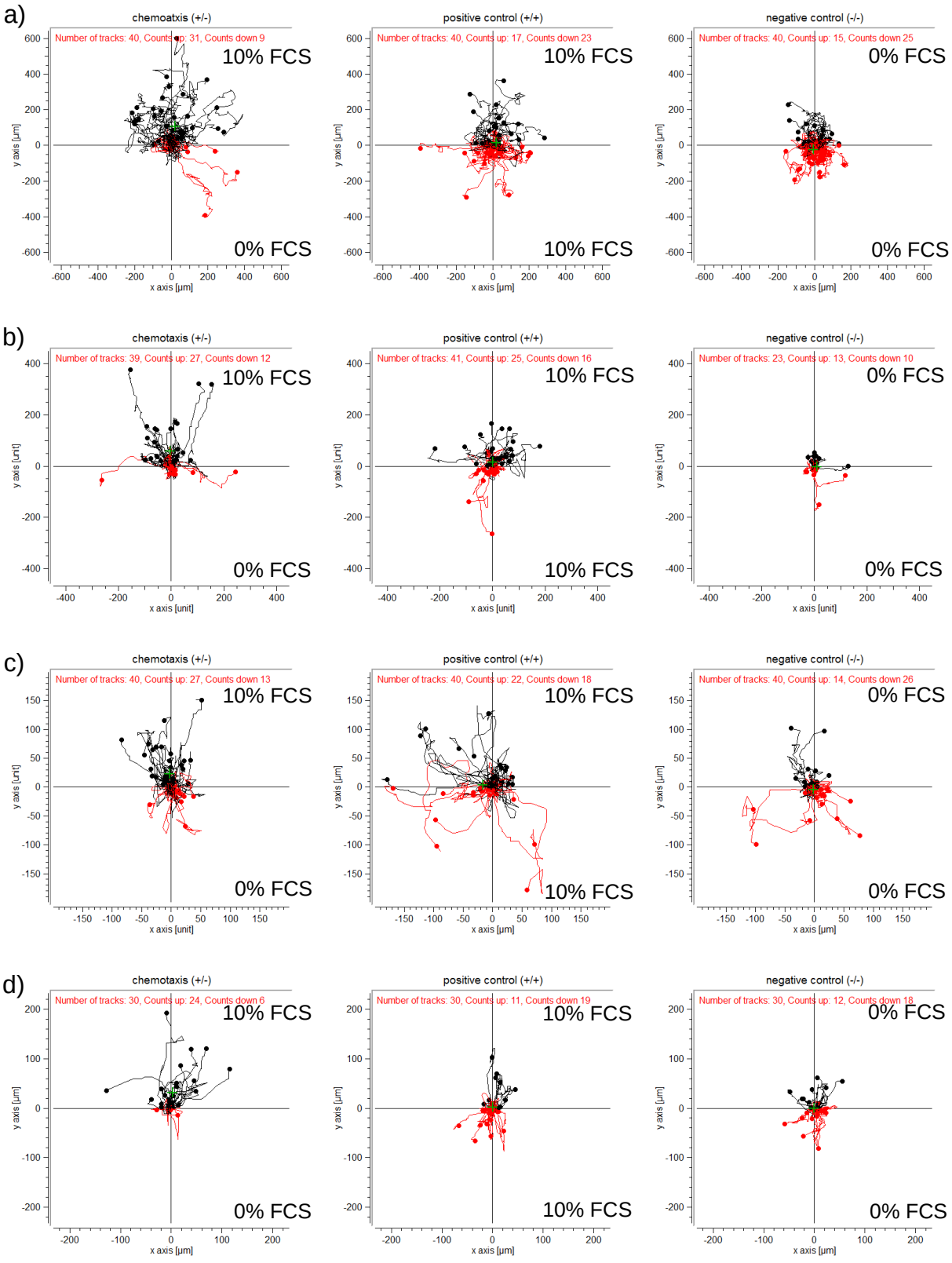

Figure 3 Representative cell trajectory plots of HUVEC cultured on a 2D surface (a), in a 1.5 mg/ml bovine collagen type I gel (b), in a 1.5 mg/ml rat tail collagen type I gel (c) and in Matrigel (d). The results of a chemotaxis experiment (left), a positive (middle) and a negative control (right) are shown for each condition. The data shown represents the time range between time point 6 hours and 18 hours.