3D Chemotaxis Protocol with bovine Collagen I Gel for Dendritic Cells

1. General Information

This is a specialized protocol for analyzing the chemotaxis of murine dendritic cells embedded in a 3D bovine collagen matrix using the µ-Slide Chemotaxis^{3D}. More detailed information about slide handling, experimental planning and data analysis is provided in the Application Note 17 "3D Chemotaxis Assays using µ-Slide Chemotaxis ^{3D} ".

2. Equipment Needed

Detailed information about the hardware and the software needed can be found in Application Note 17. In short, the following equipment and instruments are necessary:

- Cell culture incubator (high humidity, 37°C, 5% CO_2)
- Inverted microscope (phase contrast) with time-lapse function
- Stage top incubator (37 °C, 5% CO_2).
- Optional: Motorized stage and autofocus (x,y,z) to observe all 3 chambers and up to 4 slides in parallel.
- Software: The ImageJ plugin "Manual Tracking" and the "Chemotaxis and Migration Tool" from ibidi.

Note: Lower magnification objectives (4x - 10x) provide a larger depth of focus and are therefore advantageous.

3. Cell Culture of Dendritic Cells

Dendritic cells (on day 8–10) should be activated over night with 200 ng/ml LPS (in cell culture medium, as described in Table 1)

Table 1 Material and Reagents Needed for the Cell Culture of Dendritic Cells.

	Reagent/Material	Concentration	Company	Order No.
Cell Culture	Dendritic cells (murine)	-	Self-prep*	-
	RPMI 1640	1x	Invitrogen	31870025
	FCS	10%	Invitrogen	10270106
	L-Glutamine	5%	Sigma-Aldrich	G7513
	Penicillin/Streptomycin	5%	Sigma-Aldrich	P4333
	GM-CSF	10-20 ng/ml*	Peprotech	315-03
Cell Activation	LPS	200 ng/ml	Sigma-Aldrich	L4516

* as published in:
Lutz, M. B. et al. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods 223, 77–92 (1999)

4. Gel Preparation, Collagen I, Bovine, 1.6 mg/ml

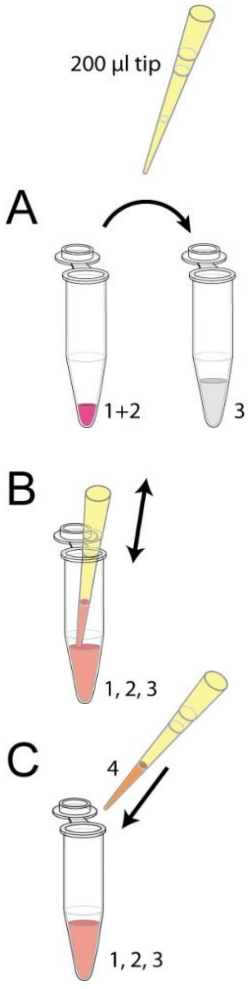
The following reagents and materials (Table 2) are necessary for the chemotaxis experiment with dendritic cells. Detailed information about the gel protocol and general handling tips are given in Application Note 26 “Fabrication of Collagen I gels”.

Table 2 Material and Reagents Needed for the Chemotaxis Experiment with Dendritic Cells.

	Reagent/Material	Concentration	Company	Order No.
Cells	Dendritic cells (murine)	9 x 10^6 c/ml	Self-prep	-
Gel Preparation	Collagen I, bovine	3 mg/ml	Advanced BioMatrix	5005-B
	$NaHCO_3$	7.5 %	Sigma-Aldrich	S8761
	10x MEM	10x	Sigma-Aldrich	M-0275
	RPMI 1640	1x	Invitrogen	31870025
Chemoattractant	CCL19	1.25 µg/ml	R&D Systems	361-MI-025
µ-Slide	µ-Slide Chemotaxis^{3D}	-	ibidi	80326

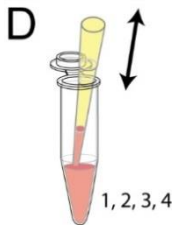
Table 3 Composition of a 1.5 mg/ml bovine collagen I gel.

V_{total} = 300 µl		
No.	Component	Volume
1	10x MEM	20 µl
2	H_2O	20 µl
3	$NaHCO_3$	10 µl
4	1x RPMI 1640	50 µl
5	Collagen I, bovine	150 µl
6	Cell suspension	50 µl
	∑	300 µl



1. Prepare a cell suspension of 9 x 10^6 cells/ml in the cell culture medium mentioned in Table 1.
2. Carefully mix the components 1, 2, 3 and 4 (Table 3) in a 1.5 ml tube. Try avoiding air bubbles.
3. Prepare 150 µl of collagen (Component 5) in a 1.5 ml tube.
4. Transfer the 100 µl mix from Step 2 into the collagen tube. Use a 200 µl pipette tip to immediately mix the larger volume (see Illustration A).
5. Mix well, but carefully (see Illustration B). It is important to avoid air bubbles during mixing!
6. Add 50 µl of the cell suspension (Step 1) into the mix (see Illustration C).

7. Mix well, but carefully (see Illustration D). It is important to avoid air bubbles during mixing!
8. Immediately afterwards, fill the mix into the channels, as described in Application Note 17.
9. Place the slide into the incubator for 45 minutes to achieve gelation. After that, collagen fibers will become visible.



5. Chemotaxis Experiment

Chemoattractant: CCL19 (1.25 µg/ml in RPMI 1640 supplemented with 10%FCS)
Chemoattractant-free medium: RPMI 1640 supplemented with 10% FCS

1. After the gelation of the gel, fill up the reservoirs with either a chemoattractant-free or chemoattractant-containing medium.
2. For the chemotaxis experiment itself (+/-), fill one reservoir with 65 µl of chemoattractant-containing medium and the other with 65 µl of chemoattractant-free medium.
3. For a control experiment (-/-), fill both reservoirs with 65 µl of chemoattractant-free medium.
4. Close the slide according to Application Note 17. The slide is now ready for video microscopy.
5. Put the slide into the stage top incubator.
6. Focus on the center of the gel and acquire images for 4 hours with a time-lapse interval of 2 minutes.

Observation on the micrsocope: Because of the 3D environment, not all cells will be perfectly in focus, depending on the objective being used.

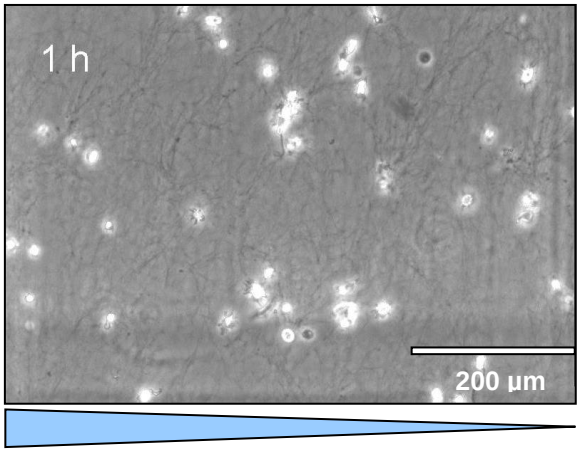


Figure 1 Bright-field image during time-lapse 1 hour after experimental set-up.

6. Experimental Results

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 4 Comparison of the migration parameters of HT1080 cells. Data for the chemotaxis experiment and the control experiment are given in the table below. CCL19 was used as chemoattractant.

	Chemotaxis Experiment (+/-)	Control Experiment
FMI^{II}	-0.65	0.004
$FMI^{\perp}$	0.07	0.05
Directness	0.66	0.15
Velocity (µm/min) ± SD	2.87+/- 1.13	2.39 +/- 0.96
p-value (Rayleigh test)	8.88×10^{-16}	0.68

A chemotactic respose could be observed when using CCL19 as chemoattractant. Both the FMI^{II} and the p-value (of the Rayleigh test) of the chemotaxis experiment indicate a directed cell migration. Contrary, random migration could be observed in the absence of CCL19 (control experiment).

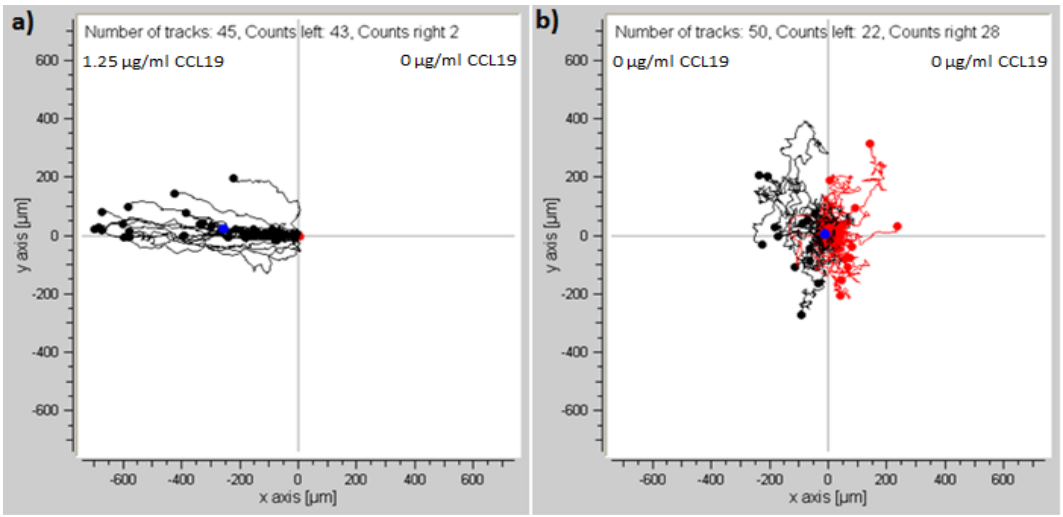


Figure 2 Representative cell trajectory plots of dendritic cells cultured in a 1.5 mg/ml collagen gel. The results of a chemotaxis experiment (a) and a control experiment (b) are presented.