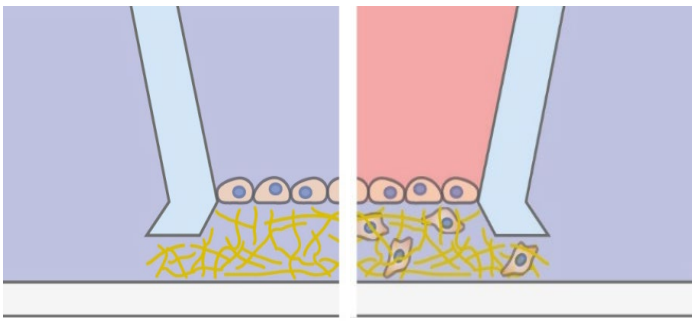
Chemotaxis-Driven Invasion Assay Using the micro-Insert 3D



Understanding cellular invasion is critical for unraveling the mechanisms of tumor progression and immune cell trafficking, both of which are central to disease development and therapeutic response. This protocol describes a chemotaxis-driven invasion assay using the micro-Inserts 3D for self-insertion, filled with a 3D collagen I matrix to evaluate the migratory behavior of Jurkat T cells.

Jurkat cells, representing fast-migrating lymphocytes, are seeded on top of the collagen gel and exposed to a serum gradient generated by different fetal calf serum (FCS) concentrations in the adjacent reservoirs. After 24 hours, cell invasion into the collagen matrix is assessed by imaging and depth quantification. Cells exposed to the FCS gradient showed significantly deeper invasion compared to the cells in a chemically homogeneous environment, independent of the absolute presence of serum.

This setup models chemotactic invasion in a three-dimensional environment and highlights the role of directional cues in promoting active migration through extracellular matrix components. The assay provides a robust and reproducible platform for studying immune cell migration and tumor microenvironment interactions under physiologically relevant conditions.

ibidi Solutions for the Chemotaxis-Driven 3D Invasion Assay

- micro-Inserts 3D for self-insertion
- µ-Plate 24 Well, ibiTreat
- Collagen Type I, Bovine

Related Documents

- Instructions micro-Insert 3D (PDF)
- Instructions Collagen Type I, Bovine, 5 mg/ml (PDF)
- AN 23: 3D Chemotaxis Protocol for Non-Adherent Cells in a Gel Matrix (PDF)
- AN 24: Chemotaxis of HT-1080 Cells in 2D and 3D (PDF)
- AN 26: Preparation of Collagen I Gels (PDF)
- AN 34: Chemotaxis of HUVECs in 2D and 3D (PDF)
- AN 44: Immunofluorescence Staining of HUVEC in 3D in the µ-Slide Chemotaxis (PDF)

1 Materials

1.1 Reagents and Buffers

- micro-Inserts 3D for self-insertion (80499, ibidi GmbH)
- µ-Plate 24 Well, ibiTreat (82426, ibidi GmbH)
- Collagen Type I, Bovine (50303, ibidi GmbH)
- Jurkat cells (ACC 282, DSMZ GmbH)
- RPMI 1640 medium (21875034, Gibco, Thermo Fisher Scientific)
- Fetal calf serum (FCS, 10270106, Gibco, Thermo Fisher Scientific)
- 10× RPMI 1640 (R1145, Sigma)
- NaOH in ultrapure H_2O, 1 M
- $NaHCO_3$ 7.5% (S8761, Sigma)
- Sterile, ultrapure water

1.2 Equipment

- Cell culture incubator (humidified, 37°C, 5% CO_2)
- Standard cell culture equipment (sterile working bench, cell detachment kit, culture flasks, pipets with suitable tips, etc.)
- Cooled aluminum beads or ice

2 Procedure

For one experiment, prepare three different samples, each in triplicate:

1. Sample with chemoattractant gradient (-/+). Apply the chemoattractant (in our case 10% FCS) only in the outer well of the micro-Insert 3D.
2. Sample with a homogeneous distribution of chemoattractant (+/+): 10% FCS is present in the whole sample, i.e. in the outer and inner well, and in the gel matrix.
3. Sample without chemoattractant (-/-): Do not add any FCS into the medium nor in the gel.

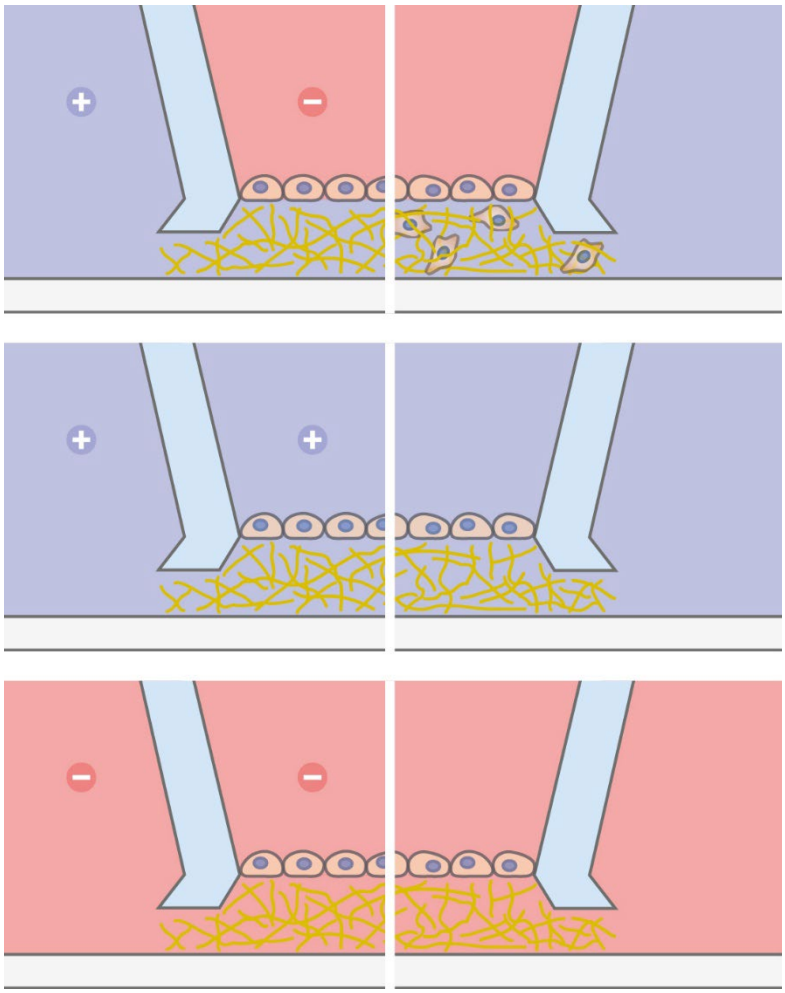


Figure 1: Exemplary drawings of the 3 conditions top (-/+), middle (+/+), bottom (-/-): The cells are seeded on top of a 3D collagen matrix in the micro-Insert 3D. Top (-/+): a chemoattractant solution is applied in the outer well of the insert, and the cells are allowed to invade into the matrix for 1–3 days (depending on the cell type). Middle (+/+): a chemoattractant solution is applied in the inner and outer well of the insert. Bottom (-/-): no chemoattractant is applied.

2.1 Sample Preparation

1. Place nine micro-Inserts 3D individually into wells of the µ-Plate 24 Well. Gently press each insert down to remove trapped air bubbles and ensure firm contact with the surface.
2. Fill the empty wells of the plate with sterile water or PBS to minimize evaporation during incubation.

2.2 Collagen Gel Preparation

Please read the Collagen Type I Instructions before starting the gel preparation.

You will need two collagen gel mixtures for this experiment:

- Without serum (for -/+ and -/- samples)
- With 10% fetal calf serum (FCS) (for the +/+ sample)

Important Note: Pipetting the Collagen Gels

Always use precooled pipet tips (4°C) for pipetting the gels.

For the preparation of collagen I gels, reverse pipetting is recommended for all steps due to the high gel viscosity. Press the pipet to the second pressure point and fill the complete pipet tip with gel. Dispense the gel only until the first pressure point is reached. This leaves a residual amount of gel in the pipet tip to be discarded, but the volume is much more accurate. Alternatively, you can use pipets designed for high viscosity solutions. Among others, we recommend Eppendorf Visco Tips or Gilson Microman E.

Note that even at 4°C, the gel mixture can be used for a maximum of 5 minutes before partial gelation occurs.

1. Prepare the collagen mixtures (1.5 mg/ml bovine collagen, with or without 10% serum) according to Table 1. Add the compounds in the order listed in Table 1 and mix thoroughly with a pipet, working on ice to slow down the gelation.
2. Apply 20 µl of the mixture to the inner well of each micro-Insert 3D. To ensure a flat collagen layer, hold the pipet straight in the middle of the well.
3. Close the plate with the lid and allow the gel to polymerize in the incubator for approximately 30 minutes.

Note: For easy calculation of different collagen concentrations, simply use the ibidi Collagen Calculator.

Table 1: Pipetting scheme for 300 µl of 1.5 mg/ml collagen I matrix with and without FCS.

	Gel without FCS (-/-, -/+)	Gel with FCS (+/+)
10x RPMI medium	20 µl	20 µl
NaOH (1 M)	9 µl	9 µl
H_2O	75.7 µl	75.7 µl
$NaHCO_3$ (7.5%)	5.3 µl	5.3 µl
1x RPMI medium (serum-free)	100 µl	70 µl
FCS	-	30 µl
Collagen I, bovine (5 mg/ml)	90 µl	90 µl

2.3 Cell Seeding

1. Harvest the cells as usual during gel polymerization.
2. Prepare two cell suspensions, at a concentration of 3 × 10^5 cells/ml:
    - Cell suspension 1: in serum-free medium (for the -/- and -/+ samples)
    - Cell suspension 2: in medium with 10% FCS (+/+)
3. Remove the plate from the incubator and verify gel polymerization under the microscope. If the matrix is not fully polymerized, incubate for an additional 10–15 minutes.
4. Fill the outer well of each micro-Insert 3D with 250 µl culture medium as follows:
    - Serum-free medium for the -/- samples
    - Medium with 10% FCS for the -/+ and +/+ samples
5. Apply 25 µl of the respective cell suspension on top of the gel matrix in each well. Avoid touching the gel matrix with the pipet tip.
6. Close the plate with a lid and return it to the incubator. Allow the cells to invade the gel for 24 hours.

3 Imaging

1. After 24 hours, record the invasion of the cells in the gel matrix using phase contrast microscopy.
2. For each micro-Insert 3D, select three random XY positions in the middle of the insert, where the collagen layer is flat. Acquire a z-stack at each position, starting from the top of the gel (where most of the cells are in focus) down to the bottom of the well, with a z-step size of 13 µm.

Note: The z-interval between consecutive images should be approximately equal to the diameter of a single cell (approximately 8–15 µm) to ensure that all cells are captured in the final image stack.

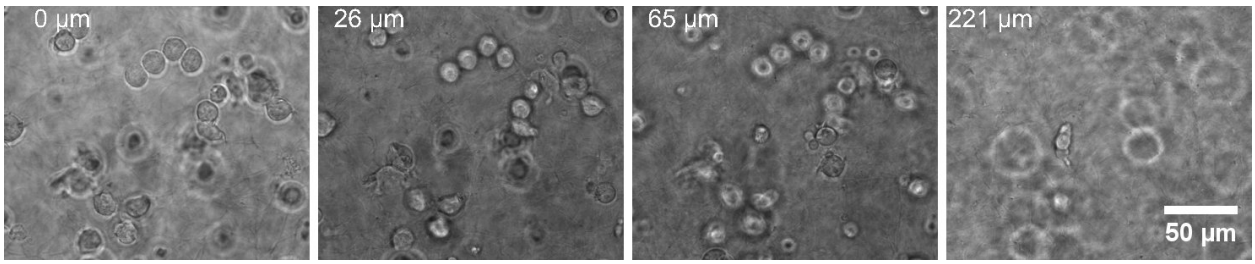


Figure 2: Representative frames from a z-stack showing Jurkat cells invading the gel matrix under FCS gradient (-/+) conditions for 24 hours. Phase-contrast images were acquired at different z-planes at a single position within the micro-Insert 3D. The first image (0 µm) shows cells located on top of the gel matrix. In subsequent images, only cells that have invaded to the indicated depth are in focus. The image was acquired using a Zeiss Axio Observer Z1 microscope with a 40× LD Plan-Neofluar objective.

4 Evaluation and Results

To evaluate invasiveness in response to the chemoattractant FCS, calculate a depth score for each well, reflecting the invasion depth into the matrix based on the number of cells in focus in individual z-plane images.

The invasion index is calculated by multiplying the sum of the number of cells (in focus) at each depth h (n_h) by the corresponding depth h (in µm), relative to the total number of cells in all depths (N):

$$Invasion\ index = \frac{\sum(n_h \times h)}{N}$$

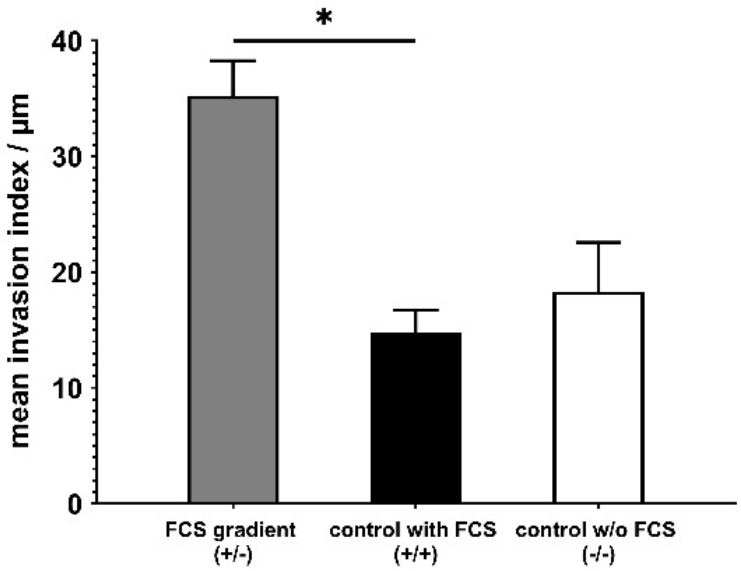


Figure 3: Chemotaxis-driven invasion of Jurkat cells in FCS gradient. The bars show the mean invasion index. Data represent mean +SEM (n = 2 independent experiments, each performed in triplicate) after 24 hours. The cells exposed to the FCS gradient invaded significantly deeper into the collagen matrix than cells in the samples with homogeneous FCS concentration. Note: In this example, cells extending across multiple slices were counted only once, in the slice where the central plane of the cell was in focus.

5 Summary

This chemotaxis-driven invasion assay using the micro-Insert 3D system provides a reliable and physiologically relevant method for quantifying immune cell migration in a 3D collagen matrix. The observed differences in invasion depth under varying serum conditions underscore the importance of directional cues in cellular motility. The protocol is well-suited for comparative studies of chemotactic behavior and can be adapted to other cell types or chemoattractants to explore diverse aspects of cell invasion and microenvironmental interactions.