Wound Healing Assay with the ibidi Culture-Insert 2 Well in a µ-Plate 24 Well

1 General Information

Cell migration plays a central role in many complex physiological and pathological processes. The wound healing assay is a simple method to study cell migration *in vitro*. The assay is based on the observation that, cells on the edge of an artificially created gap in a confluent monolayer will migrate until new cell-to-cell contacts are established. The ibidi Culture-Insert 2 Well provides a complete solution for wound healing experiments, requiring only a few steps to go from sample preparation to image analysis.
ibidi offers the Culture-Insert 2 Well in different product variations: Culture-Inserts placed in µ-Dishes, high and low (ibidi, 81176 and 80206); Culture-Inserts placed in a µ-Plate 24 Well (ibidi, 80241); and Culture-Inserts for self-insertion into a culture vessel of you choosing (ibidi 80209). While the Culture-Inserts in µ-Dishes are recommended for single experiments, the Culture-Inserts in the µ-Plate 24 Well offer a convenient high-throughput option.
This Application Note is a detailed protocol for analyzing the migration behavior of MCF-7 cells using the ibidi Culture-Insert 2 Well 24. The influence of five different concentrations of the human epidermal growth factor (hEGF) on the migration behavior was tested in parallel and compared to the control condition.
More detailed information about experimental planning and data analysis is provided in Application Note 21 “Wound Healing Assay” and in Application Note 30 “Data Analysis of Wound Healing Assays”.

2 Material

- Cells: MCF-7 (ATCC: HTB-22; DSMZ: ACC115)
- ibidi Labware: Culture-Insert 2 Well 24, ibiTreat (ibidi, 80241)
- Cell culture surface: ibiTreat
- Cell culture medium: RPMI (Sigma, R8758) + 10% FCS (Sigma, F0804)
- Cell dissociation solution: Trypsin-ETDA (Sigma, 59418C)
- Growth factor: human epidermal growth factor (hEGF) (Promokine, C-60170)
- Sterile tweezers
- Inverted microscope, preferably with an automated image acquisition system and stage top incubator for live cell imaging

3 Experimental Workflow

The influence of hEGF on the migration behavior of MCF-7 cells was tested in this experiment. Five different hEGF concentrations were used and the migration behaviors were compared to control cells that were not treated with the hEGF.

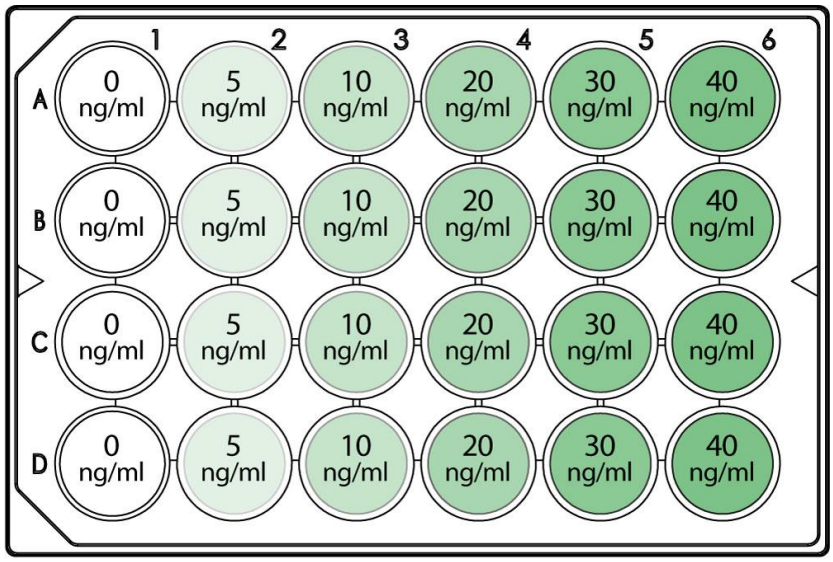

Figure 1 Experimental Set-Up: Five different hEGF concentrations (green) were tested (5, 10, 20, 30, 40 ng/ml). Untreated cells (white) served as the control. Four technical replicates were performed for each condition.

3.1 Step 1: Cell seeding

The correct seeding concentration is a critical parameter, as a confluent monolayer should be reached after 24 hours. Pre-experiments are necessary to define the best concentration for the used cell line.

1. Remove the protective foil attached to the bottom of the µ-Plate (Figure 2, A).
2. Prepare the cell suspension as usual. It is recommended to include a centrifugation step to remove dead cells and cell debris. Adjust the MCF-7 cell suspension to a cell concentration of $5x10^5$ cells/ml.
3. Apply 70 µl of the cell suspension into each well of the Culture-Inserts 2 Well (Figure 2, B). Avoid shaking the µ-Plate as this will result in an inhomogenous cell distribution.
4. Incubate your cells at 37 °C and 5% CO_2 for at least 24 hours.

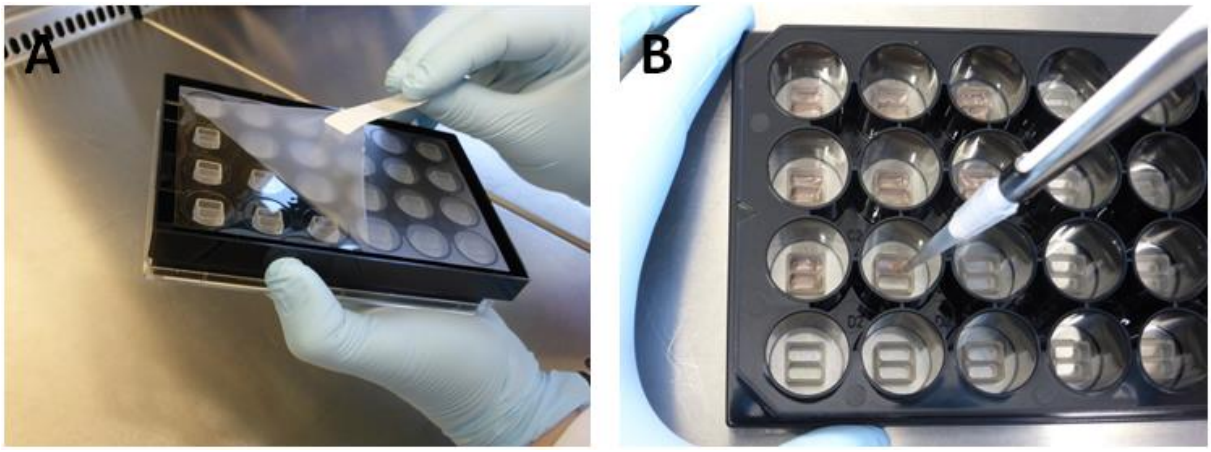

Figure 2 Remove the protective foil before starting the cell seeding step (A). Fill 70 µl of cell suspension into each well of the Culture-Inserts 2 Well (B).

3.2 Step 2: Gap formation

The use of the µ-Plate 24 Well allows testing of five different hEGF concentrations and a control condition in parallel. Four technical replicates can be performed for each experimental condition (Figure 1).

1. Check the cell density after 24 hours under the microscope. If a confluent cell monolayer is not achieved after 24 hours, place the µ- back in the cell culture incubator for another couple of hours. Check the confluence regularly.
2. Add the growth factor to the cell culture medium to obtain the following concentrations: 0, 5, 10, 20, 30 and 40 ng/ml EGF.
3. Gently remove the Culture-Inserts 2 Well with sterile tweezers. To remove the Culture-Insert grab one corner as shown in figure 3.
4. Wash the cell layer with cell-free medium or PBS to remove cell debris and non-attached cells.
5. Carefully aspirate the cell-free medium or PBS.
6. Pipette 1 ml of cell culture medium into each well of the 24 Well Plate.

Figure 3 Removing the Culture-Inserts 2 Well using sterile tweezers.

3.3 Step 3: Acquisition of microscopy images

We recommend recording a time-lapse video to determine the time dependency and the characteristics of cell migration.

1. Place the µ-Plate 24 Well on the microscope and define the positions of all 24 wells.
2. Start the observation process by taking images several times throughout the following hours. Images are taken every 30 minutes over 24 hours for the data shown in the next section.

4 Results

The microscopic images have to be analyzed to obtain information about the migration characteristics of the cultured cells. Analyzing the changes in the cell covered area over time will allow you to determine the cell velocity. A detailed description about data analysis is found in Application Note 30 “Data Analysis of Wound Healing Assays”.

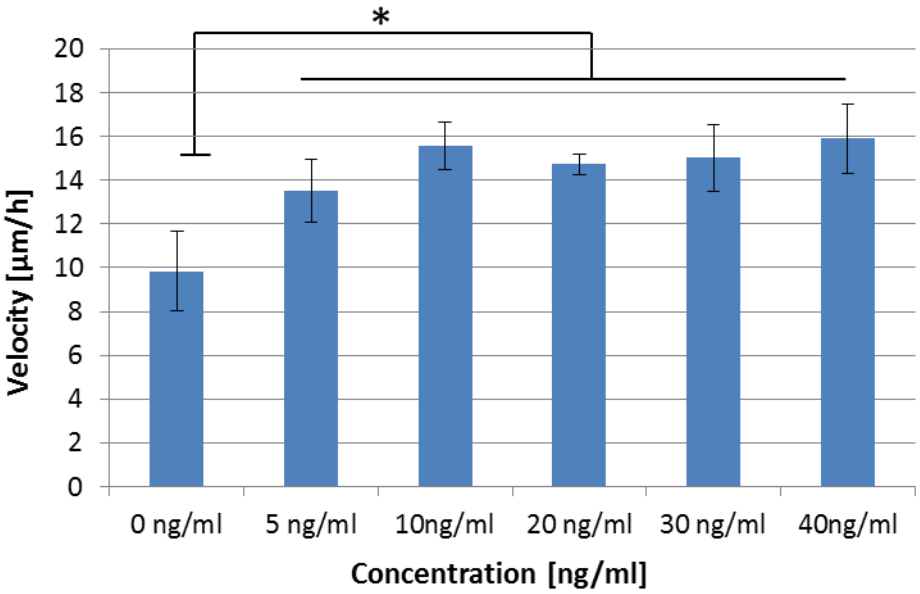

Figure 4 Comparison of the influence of hEGF on the migration behavior of MCF-7 cells. Four different EGF concentrations were tested and compared to the control experiment.

Using the Culture-Insert 2 Well 24 allows testing six different (or more) conditions in parallel. The influence of five different hEGF concentrations (four replicates each) was analyzed by comparing the cell velocity to the velocity of the control condition. The experimental data revealed that hEGF increased the cell velocity of MCF-7 cells compared to the control group. Concentrations of hEGF > 10ng/ml showed no further increase in the cell velocity, as observed in Figure 4.