ibidi® cells in focus

Wound Healing Assay

with the ibidi Culture Insert in a µ-Dish 35 mm

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*. This assay is based on the observation that, upon the creation of a new artificial gap on a confluent cell monolayer, the cells on the edge of the created gap will start migrating until new cell-cell contacts are established. The ibidi Culture-Insert provides a complete solution for wound healing experiments, requiring only a few steps to go from sample preparation to image analysis.
This Application Note is a detailed protocol for analyzing the migration behavior of MCF-7 cells using the ibidi Culture-Insert. More detailed information about experimental planning and data analysis is provided in Application Note 30 "Data Analysis of Wound Healing Assays" and in the Instructions "Instruction Culture Insert".

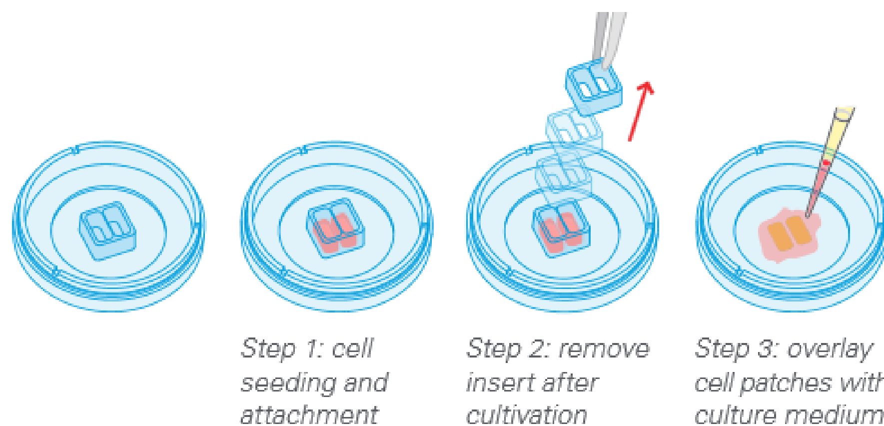

Figure 1 Experimental workflow for performing a wound healing assay using the ibidi Culture-Insert.

Placed on a cell culture surface, the ibidi Culture-Insert provides two cell culture reservoirs, each separated by a 500 µm wall. Filling cell suspension in both reservoirs allows cell growth in the designated areas only. Removing the Culture-Insert after appropriate cell attachment creates a cell-free gap of approximately 500 µm. Microscopy is used to evaluate the wound healing process. Depending on your focus of interest, this can either be done by using video microscopy, or by observing images at distinct time points. Measuring the changes in cell covered area allows quantifying the speed of wound closure and provides cell migration characteristics.

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2. Material

- Cells: MCF-7 (ATCC: HTB-22; DSMZ: ACC115)
- ibidi Labware: Culture-Insert in µ-Dish 35 mm, high (ibidi, 80206)
- Cell culture surface: ibiTreat
- Cell culture medium: RPMI (Sigma, R8758) + 10% FCS (Sigma, F0804)
- Cell dissociation solution: Trypsin-ETDA (Sigma, 59418C)
- Sterile tweezers
- Inverted microscope, preferable with an automated image acquisition system and stage top incubator for live cell imaging

3. Experimental Workflow

3.1. Step 1: Cell seeding

In order to establish a reliable data acquisition system, you have to define the experimental parameters before starting the experiment. This includes for example the choice of the right cell seeding density. We recommend using a density that leads to a confluent cell after 24 hours.

1. Remove the protection foil attached to the bottom of the µ-Dish.
2. Prepare your cell suspension as usual. It is recommended to include a centrifugation step to remove dead cells and cell debris. Adjust the cell suspension to a cell concentration of $3x10^5$ cells/ml to obtain a confluent cell layer after 24 hours.
3. Apply 70 µl cell suspension into each well of the Culture-Insert. Avoid shaking the µ-Dish 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.

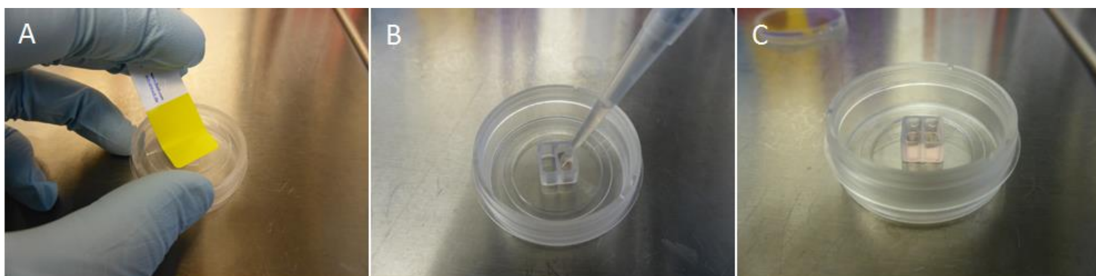

Figure 2 (A) Removal of the protection foil is necessary before starting the experiment. (B, C) Seeding cells in the ibidi Culture Insert.

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3.2. Step 2: Gap formation

A confluent cell layer is a prerequisite for starting this assay. Fresh medium is added after the removal of the Culture-Insert. If necessary, supplement your medium with inhibiting or enhancing substances for evaluating their effects on the migration behavior of the cells.

1. Check the cell density after 24 hours under the microscope. In case that no confluent cell layer is reached after 24 hours place the µ-Dish again in the cell culture incubator for another couple of hours. Check the confluence regularly.
2. Gently remove the Culture Insert with sterile tweezers. For removing the Insert grab one corner as shown in figure 3.

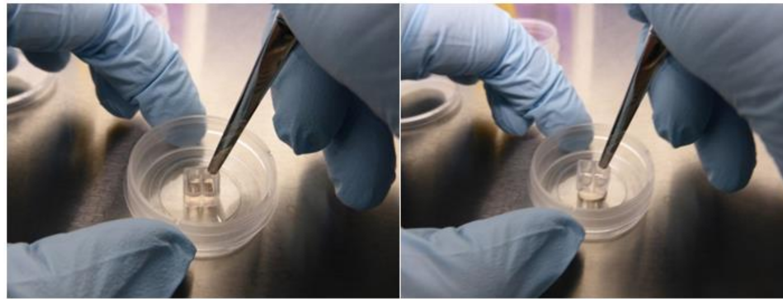

Figure 3 Removal of the ibidi Culture Insert using sterile tweezers.

3. After removing the Insert, check whether your cell layer is still attached to the surface of the µ-Dish.
4. Wash your cell layer with cell-free medium or PBS to remove cell debris and non-attached cells.
5. Fill the µ-Dish with cell-free medium applying the recommended volume of 2 ml.

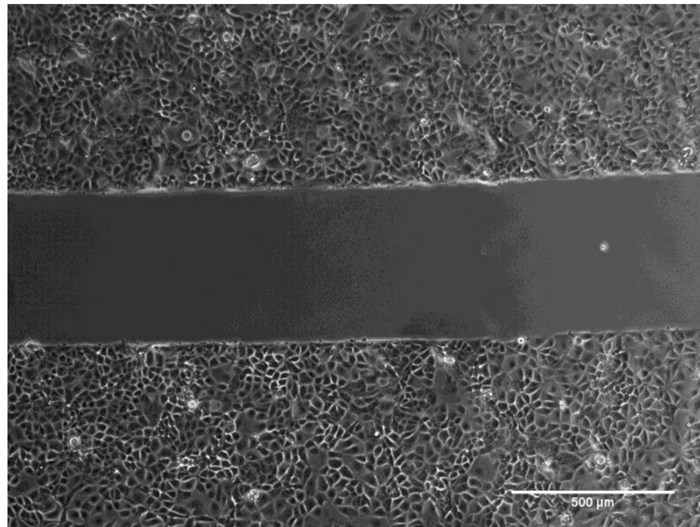

Figure 4 Cell free gap created by the ibidi Culture Insert.

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3.3. Step 3: Acquisition of microscopy pictures

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

1. Place the dish on the microscope and move it until you have the gap and both cell fronts captured in the image. The orientation of the wound area is not critical, but needs to be horizontal or vertical.
2. Start the observation process by taking images several times throughout the following hours.
3. Time lapse measurements for MCF-7 cells cultured on the ibiTreat surface were performed for 20 hours with a time interval of 30 minutes.

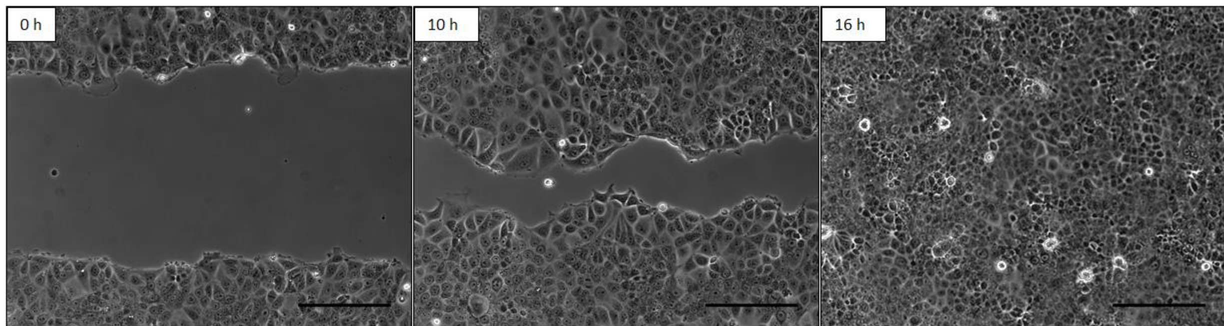

Figure 5 Time lapse measurement of a migration assay using MCF-7 cells. (Scale bar: 200 µm)

3.4. Step 4: Quantitative image analysis and interpretation

The microscopic pictures have to be analyzed for obtaining information about the migration characteristics of the cultured cells. This can either be done by hand using an image processing software, or by automated image analysis using WimScratch. A detailed description about the establishment of experimental parameters, data analysis and interpretation can be found in Application Note 30 “Data Analysis of Wound Healing Assays”.

The example data shown here was analyzed using the WimScratch software. The automated image analysis detects the cell covered area. Plotting the cell covered area against the time showed the process of gap closure. At the beginning, just after removing the Culture-Insert, occurred a lag phase of about one hour. This was followed by a linear phase which can be used to characterize the migration speed of wound closure. When reaching 100% optical confluence (after 11 hours), the curve came to saturation.
An average cell front velocity of 13 µm/h could be detected for MCF7 cells that were cultured on the ibiTreat surface in RPMI supplemented with 10% FCS.

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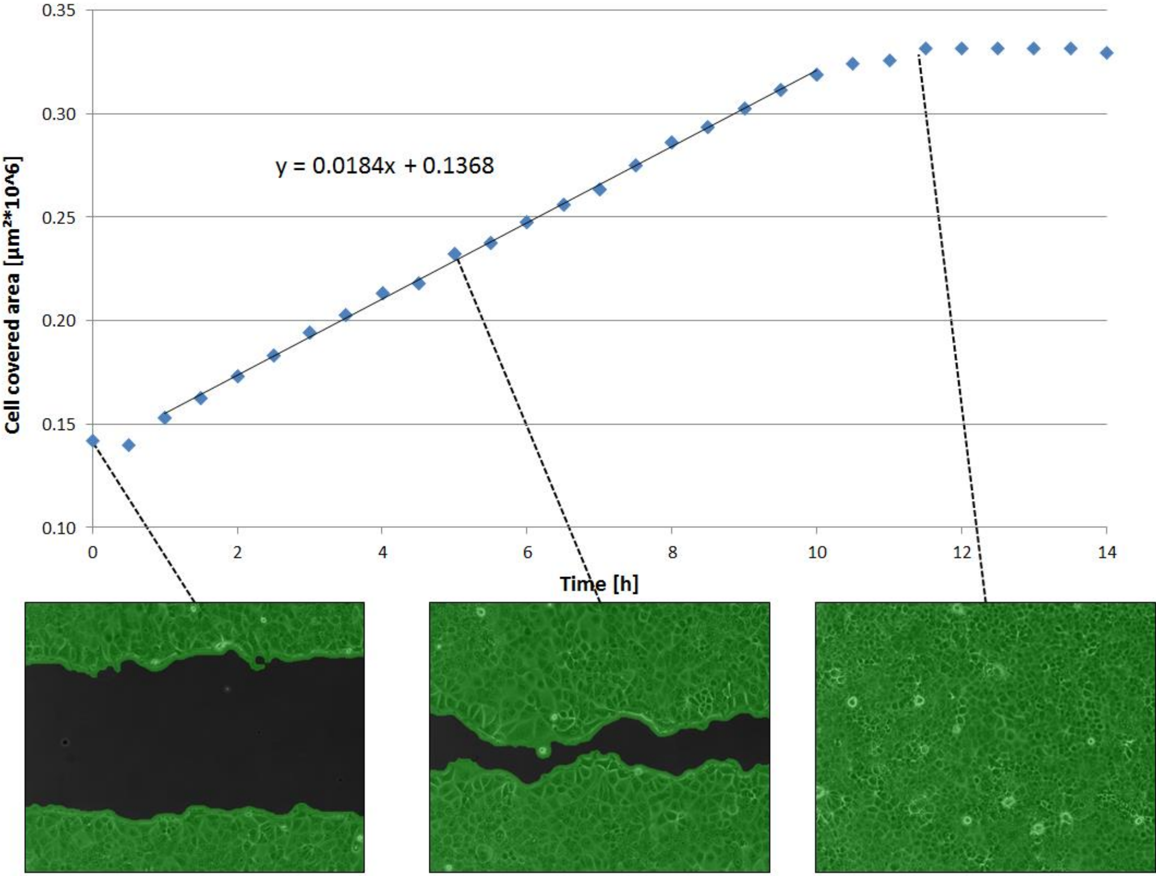

Figure 6 Quantitative image analysis of a wound healing experiment.

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