Applied BioPhysics
Quantifying Cell Behavior

zenCELL owl

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Application Note: Scratch Assay

Cell migration analyzed with the 24-channel microscope zenCELL owl

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Introduction

Cell migration is involved in many complex physiological and pathological processes. It plays a central role in basic biological events such as tissue generation, renewal and repair, wherein old or damaged cells are replaced by the migration of newly formed cells. Cell migration also occurs in mediating immune responses and wound healing. Undesirable cell migration events take place in the progression of diverse diseases, e.g. cancer invasion and metastasis. For these reasons, cell migration has to be controlled carefully to maintain tissue integrity and homeostasis.

A useful tool to analyze cell migration is the wound healing assay. It is a standard method to study cell migration in two dimensions *in vitro*. A cell-free gap is created in a cell monolayer either by direct manipulation or by physical exclusion. Subsequently, the dynamics of the migration into the cell-free area are monitored and quantified. This provides information about cell migration characteristics and wound healing rate. Additionally, 2D-migration assays can be used for screening the effects of substances on their wound healing and regenerative potential on mammalian cells.

The most common approach to insert a gap in a confluent monolayer is the scratch assay. In order to set the scratch onto the cell monolayer a pipette tip, a needle or another sharp tool is used. Advantages are the low cost and easy handling. Another way to create a cell-free gap is physically excluding the cells by using inserts that prevent cells to grow in a defined region. This method generally causes minimal damage to the remaining cells compared to the direct mechanical damage of the monolayer.

In this application note, a wound healing assay was performed. Cell migration was imaged using the zenCELL owl incubator microscope (Figure 1). A software especially developed for the zenCELL owl determines the cell coverage of the substrate surface of the section (1,2 mm x 0,9 mm) magnified by the microscope via a real-time data analysis. The current cell count can also be determined. Simultaneously, the microscope documents the quality of each of these individual cell cultures using image recordings. Analyses were performed in all 24 wells of a standard cell culture plate at the same time in intervals of 10 minutes over the measurement period.

Figure 1: zenCELL owl. 24-channel microscope with 24-well cell culture plate.

Cell culture and scratch Assay

Cells from the L929 mouse fibroblast cell line were seeded in 24-well plates at a density of 120000 cells/well and grown for 24 hours in standard Dulbecco´s Modified Eagle´s Medium (DMEM) supplemented with 5% FBS and 50 µg/mL Gentamycin. Then, a scratch (Figure 2) was inserted on the confluent cell monolayer using a 10 µL pipette tip in each well and rinsed with Phosphate-buffered saline twice. After the addition of fresh cell culture medium, the 24-well cell culture plate was inserted in the zenCELL owl microscope and grown for 24 hours. The incubation environment was maintained at 37°C and a constant 5% CO_2 in air in a humidified cell incubator. Pictures were taken in intervals of 10 minutes and the cell coverage was calculated from these values.

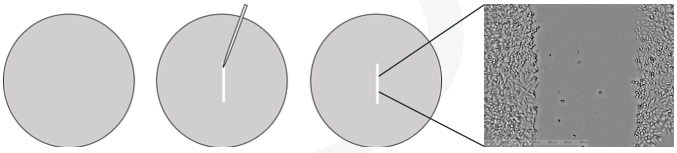

Figure 2: Scratch assay.
a) confluent cell monolayer.
b) Gap is inserted by a pipet tip.
c) Cell-free gap (width 600 µm) before cell migration.

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Results:

The microscope pictures show a nearly complete closure of the gap within 24 hours after inserting the scratch (Figure 3). The cells spread and moved from the edges of the gap into the free space. Sporadic cell divisions are visible in the pictures. To prevent that cell proliferation could interfere with the measurement of migration, proliferation could be inhibited by addition of drugs like Actinomycin C or deprivation of growth factors (e.g. by serum starving). The data of Cell Coverage correlate with the pictures of the wound healing process (Figure 4). The cell coverage of the area rises from 50% at the beginning to 90% within 24 hours of the experiment.

Calculating the Relative Gap Area (Figure 5), together with the equation of a polynomial fitting curve that describes the data, from the given Cell Coverage data allows the determination of the time-point of half gap closure ($t_{1/2\ Gap\ Closure}$).

This Application Note shows the potential of the zenCELL owl microscope for migration assays. The zenCELL owl microscope provides the opportunity to visualize and monitor the migration of cells at every desired timepoint of the wound healing process. The zenCELL owl is a 24-channel microscope designed for fast and automated cell culture microscopy. The combination of stability and compact size makes it perfect for use in incubators. The modular design allows flexible configurations to ensure a secure analysis of biological samples.

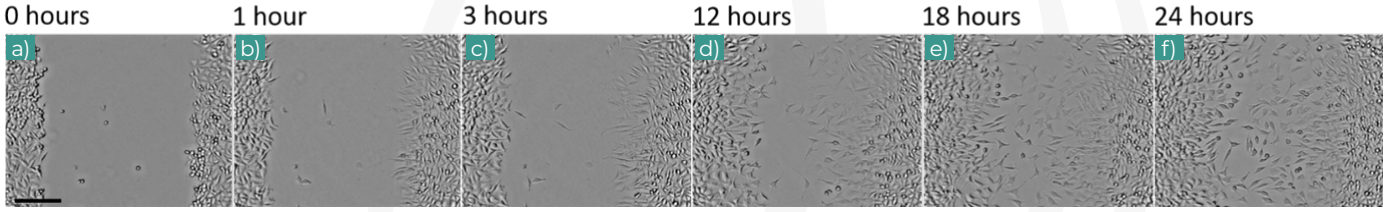


Figure 3: Gap closure over 24 hours. The images were taken with the zenCELL owl microscope.
a) The gap was inserted in a confluent monolayer using a pipet tip.
b) 1 hour after scratching the cell monolayer, the cells start to migrate into the cell free area.
c-e) Within 24 hours, the cell migration results nearly in a full f) closure of the gap. Scale bar: 200 µm.

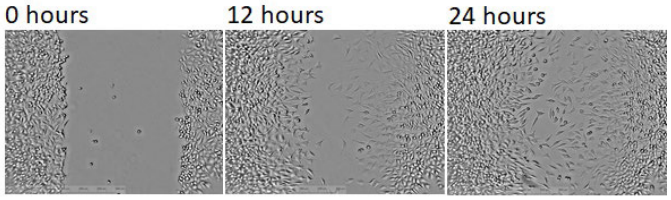
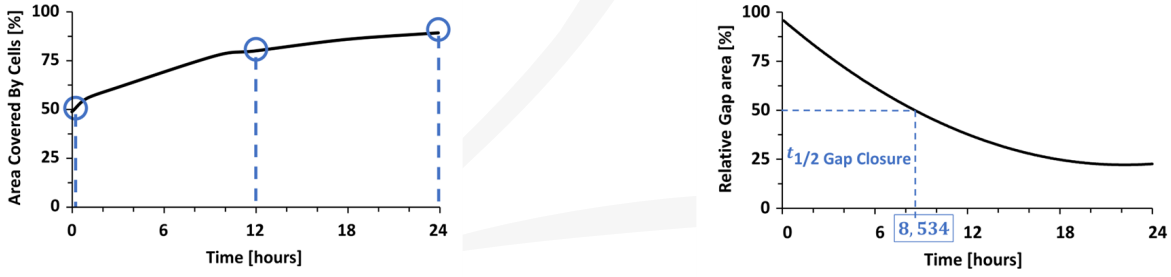
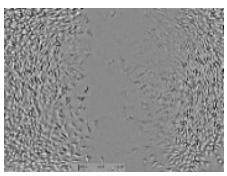


Figure 4: Changes in Cell Coverage during wound healing process. The zenCELL owl pictures visualize the progress of wound healing at every time point.





$t_{1/2\ Gap\ Closure}$: 8,534 hours

Figure 5: Relative Gap Area. The data of Cell Coverage were used to calculate the Relative Gap Area. Plotted as a function of time, the time-point of half gap closure ($t_{1/2\ Gap\ Closure}$) can be determined from this graph.

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