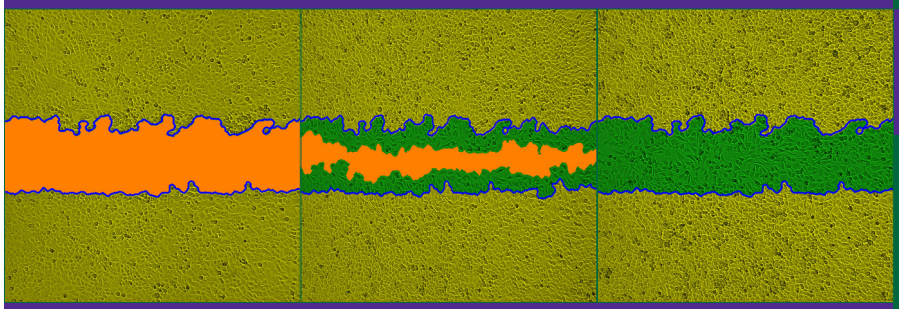

TECHNICAL NOTE

Live-Cell Imaging

Kinetic Scratch Wound Assay on the MuviCyte Live-Cell Imaging System

Introduction

Cell migration is a central process in the development and maintenance of multicellular organisms and plays an important role in the progression of various diseases including cancer. To study cell migration dynamics or to identify drugs affecting cell migration, the wound healing or scratch wound assay is the most prominent and popular *in vitro* method. As experimental procedures used in scratch wound assays are highly variable and lack standardization, assay results from different experiments or sources are often difficult to compare.[1]

Here we present a kinetic, label-free, live-cell scratch wound assay, using the MuviCyte™ live-cell imaging system, which is robust and reliable as a result of:

- Simultaneous creation of 96 uniform and reproducible scratches in cell monolayers
- Automatic detection and positioning of wounds during time-lapse acquisition
- Automated quantification of six parameters of wound closure over time

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Materials and Methods

Cell Culture and Scratch Wound Creation

HeLa and MCF-7 cancer cells were plated at 1.4E4 and 8E4 cells per well in 100 µl growth medium into a 96-well PerkinElmer ViewPlate-96. Please refer to Table 1 for materials used in this application.

Table 1. Materials for scratch wound application.

Growth Medium	Phenol red-free RPMI-1640 (Sigma, #R7509) 2 mM L-glutamine (Sigma, #G7513) 10 % FCS (Sigma, #F9665)
Inhibitor	Cytochalasin D (Sigma, #C8273)
Inducer	PMA (phorbol 12-myristate 13-acetate) (Sigma, #P1585)
Microplate	ViewPlate-96 Black, Optically Clear Bottom, Tissue Culture Treated, Sterile (PerkinElmer, #6005182)
Scratcher Tool	MuviCyte Scratcher (PerkinElmer, #HH40000301)
Analysis Software	MuviCyte Scratch software (PerkinElmer, #HH40000501)
Imaging Instrument	MuviCyte Live-Cell Imaging System (PerkinElmer, #HH40000000)

After 48 hours, cells reached 90 to 100 % confluency and the cell plate was inserted into the plate rack of the MuviCyte Scratcher tool under sterile conditions. Scratches were introduced with a single guided slide of the MuviCyte Scratcher (Table 1, Figure 1).

The resulting horizontal scratches are 500 µm wide and their position within the wells is slightly off-centered as cells do not always grow in the well center. To remove detached cells and debris from the wounded monolayer, wells were gently rinsed twice with 100 µl PBS. To inhibit or stimulate cell migration, fresh medium was added, containing either PMA or Cytochalasin D in varying concentrations: PMA (phorbol 12-myristate 13-acetate) from 0.3 to 300 nM in eight concentrations, and cytochalasin D from 0.03 to 2 µM in six concentrations (Table 1).

Automated Live-Cell Time-Lapse Imaging

To follow the kinetics of wound closure, a time-lapse measurement was set up on the MuviCyte live-cell imaging system, operated inside a standard cell culture incubator. Plates were equilibrated on the instrument for 15 minutes, followed by time-lapse imaging every 30 mins over a time period of 34.5 hours, using a 4x objective lens in the brightfield channel. The MuviCyte control software was set to scratch mode which enables the automated detection and centering of scratch wounds during the first cycle of a measurement series. Only this exact field of view (FOV) is then measured in all time-lapse cycles.

SCRATCHER KEY ADVANTAGES

- Precision engineered pins to create 96 uniform scratch wounds
- Compatible with PKI Viewplate 96 (Cat. 6005182) or Corning 96 TC-treated plates (Cat. 3595)
- Pins replaceable in case of damage
- Scratch position is off-centered as cells tend not to grow in the well center

Figure 1. The MuviCyte Scratcher enables the creation of uniform scratch wounds in 96-well plates.

Automated Image Analysis

To quantify the migration assay, an automated scratch wound image analysis was performed using the MuviCyte scratch software. Readouts are calculated based on the detection of the cell-free wound at the first time point and the creation of a mask applied to all subsequent time points (Figure 2).

The MuviCyte scratch software automatically calculates six key metrics of kinetic scratch wound assays – the wound confluence, wound area, wound width, relative wound density (RWD) and the speed of wound closure.

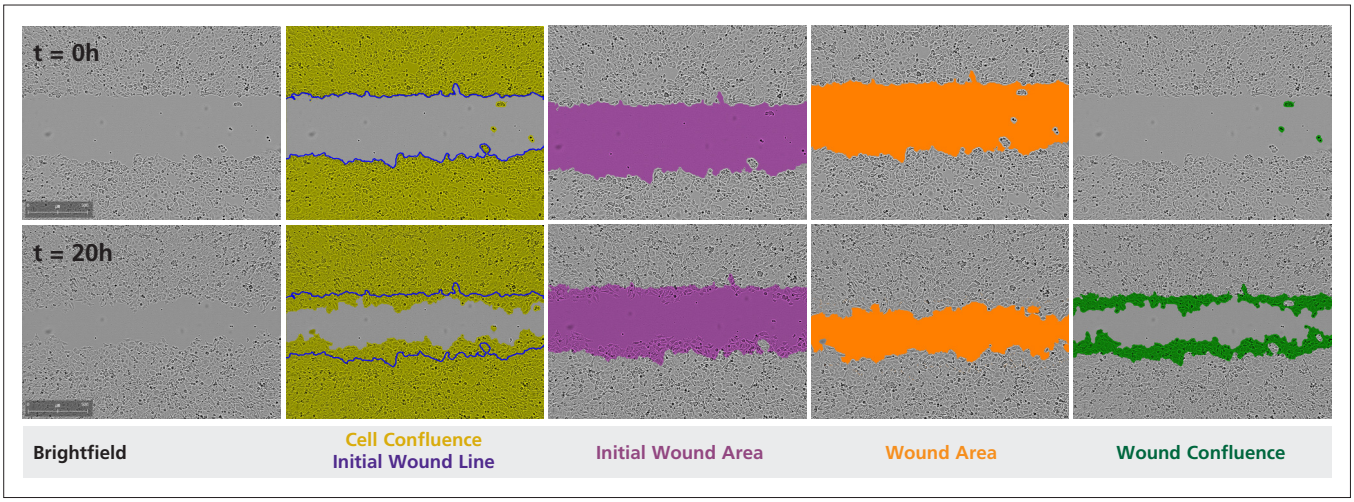

Figure 2. Visualization of the automated, label-free image analysis with the MuviCyte Scratch software. The initial scratch wound is detected and serves as mask for subsequent timepoints. The initial wound border is shown in blue, the initial wound area in pink and the wound area at a given time in orange. Yellow displays the cell area used to calculate the cell confluence in the FOV. Migrating cells growing into the wound are displayed in green.

Results

Using the MuviCyte live-cell imaging system, we studied effects of PMA and cytochalasin D on HeLa and MCF-7 cells in a classical scratch wound assay. PMA is known to speed up wound closure in epithelial cells by activating PKC (protein kinase C) subfamilies, while the fungal metabolite cytochalasin D depolymerizes the actin skeleton, thereby decreasing cell motility and migration ability.[3,4] We analyzed and confirmed the effects of both compounds using the MuviCyte scratch software. In Figure 3, representative example images of untreated and treated MCF-7 cells at time point 0 hours, 17 hours and 34.5 hours post scratch are presented, showing a strong and sensitive reaction to both compounds even at low concentrations.

The Scratch software automatically generates a plate graph showing an overview of results for the entire plate in addition to well-based graphs for more detailed analysis of individual wells. If a plate map is generated, describing the content of the plate, individual colors can be selected to represent compounds, concentrations and cell types in the graphs. Here, the readout relative wound density (RWD) in % was selected to compare the wound healing ability between wells (Figure 4). Outliers (marked in red) were identified and excluded from graphs and further analysis with a single click.

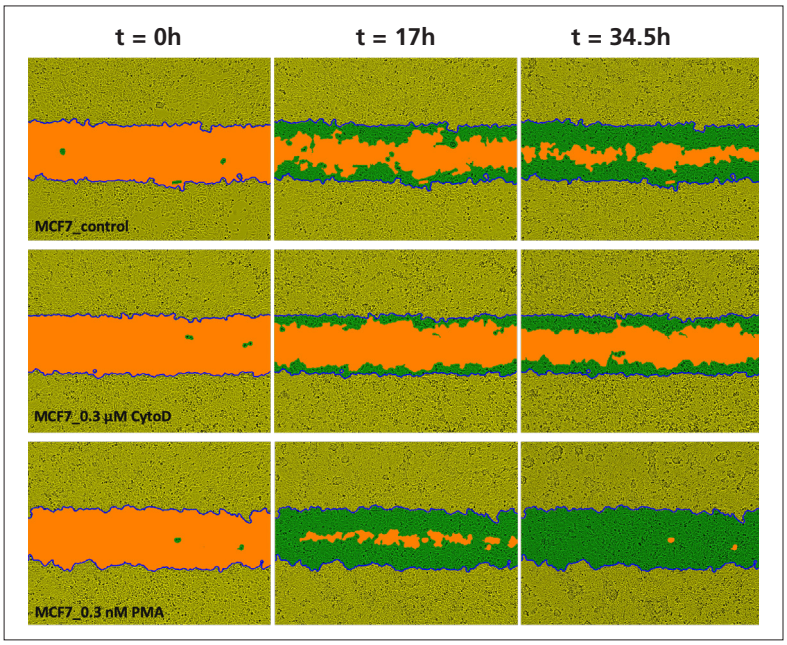

Figure 3. Wound healing in MCF7 cells is inhibited by Cytochalasin D and stimulated by PMA. Automated image analysis was done on brightfield images using the MuviCyte scratch software (Method A, Background correction activated). While untreated MCF-7 cells could not close the wound completely within a 34.5 hour time frame, PMA-stimulated cells reached confluency. Cytochalasin D inhibited cell migration into the wound even at a low concentration.

All numerical results, including mean values with their standard deviations, can easily be transferred to external software such as Excel for secondary analysis. IC_{50} values can, for example, be calculated with GraphPad Prism software, as shown here for Cytochalasin D (Figure 5). Both cell lines reacted very similarly to this compound (HeLa: IC_{50} = 0.25 µM, MCF-7: IC_{50} = 0.19 µM)

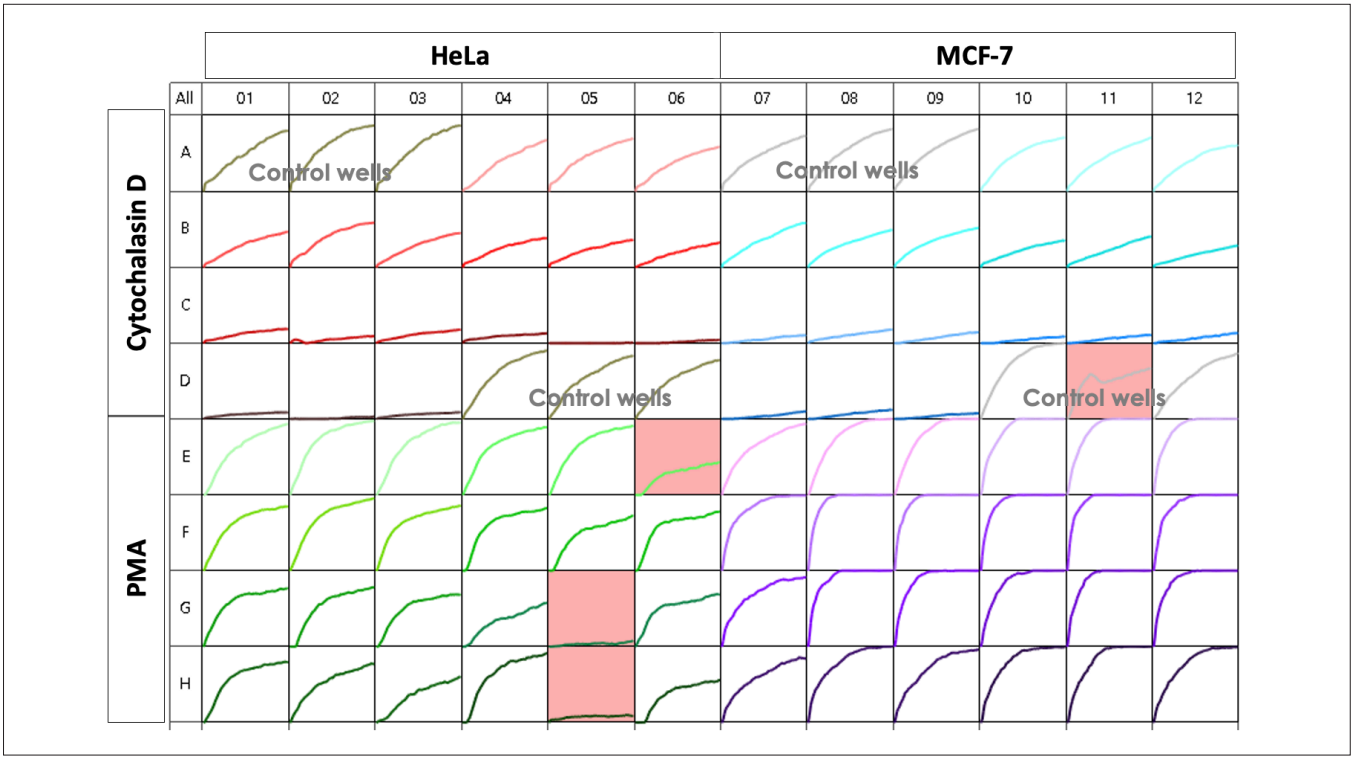

Figure 4. Plate graph of RWD (relative wound density) in % over a time period of 34.5 hours. Different colors show triplicates of treatments with cytochalasin D in six concentrations and PMA in eight concentrations. HeLa cells are on the left side (columns 1 to 6) and MCF-7 cells on the right (columns 7 to 12). Four wells were identified as outliers, marked in red and excluded from calculation of mean values and standard deviations.

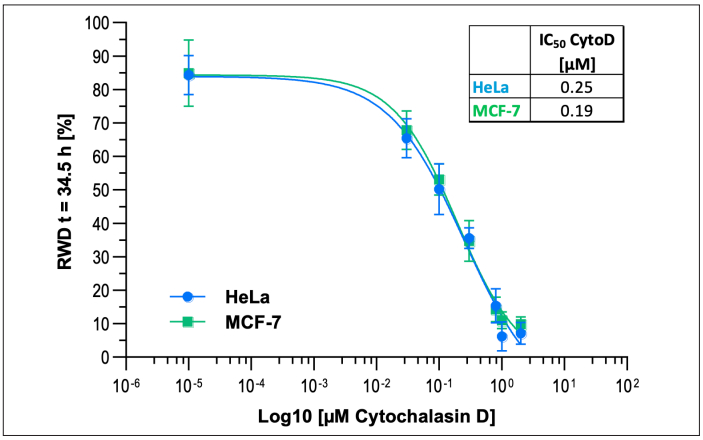

Figure 5. IC_{50} curves for cytochalasin D-induced inhibition of wound closure in HeLa versus MCF-7 cells. Generated in GraphPad Prism using exported mean RWD percentages at time point 34.5 hours (n=3). Both cell lines react similarly to the inhibitor and in a dose-dependent manner.

While the response of both cell lines to Cytochalasin D was comparable, HeLa and MCF-7 showed different responses to PMA (Figure 6). PMA stimulated wound healing in MCF-7 cells more strongly than in HeLa cells.

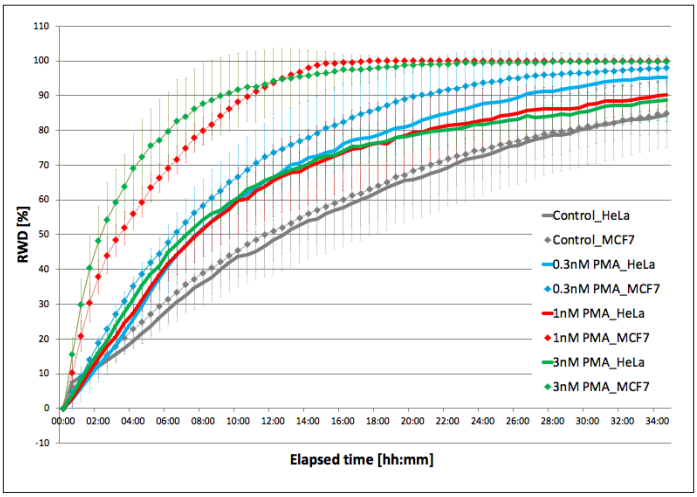

Figure 6. PMA stimulates wound healing more strongly in MCF-7 cells than in HeLa cells: MCF-7 cells were able to close the wounds after 18 hours at 1 nM PMA, while HeLa stimulation was less pronounced and resulted in RWD being only 78 % at this timepoint. Graph generated in Excel (n= 3 wells).

Conclusions

The MuviCyte live-cell imaging system, MuviCyte Scratcher and MuviCyte Scratch software together provide a robust and reproducible solution for kinetic live-cell scratch wound assays. Key benefits include:

- Creation of 96 uniform horizontal scratch wounds in cell monolayers provides reproducible assay results. Invasion can also be studied, if scratches are overlayed with biomatrices.
- Constant environmental conditions and undisturbed continuous monitoring of cells within your cell culture incubator provides robust results, without the need to fix cells at multiple time points.
- Automated wound detection in the first imaging cycle to center scratch wounds into the FOV removes residual scratch wound offsets between wells to ensure robust image analysis results. Can also be applied to manually created wounds.
- Label-free assay which avoids possible cell pertubations caused by fluorescent labeling and imaging. Image analysis is compatible with brightfield and fluorescent images, different shapes and orientations of wounds and various plate formats.
- Automatic quantification of six relevant key metrics typically used in scratch wound assays. Simple export of numerical results, images or time-lapse movies.

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