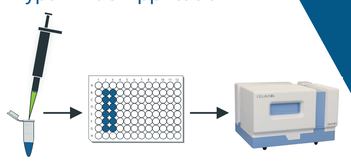
Development of a High-Throughput Wound Healing Assay to Analyze Cell Migration Using SYNENTEC's Automation System

Schaefer, W.[1]; Philipp, L.-M.[1, 2]; Guledani, A.[1]; Werdelmann, B.[1]; Stoehr, M.[1]; Schulze, K.[1]; Sebens, S.[2]; Geisen, R.[1] & Pirsch, M.[1]

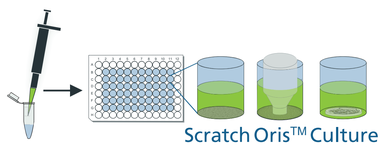
[1]SYNENTEC GmbH, Elmshorn, Germany; [2]Institute for Experimental Cancer Research, CAU + UKSH, Kiel, Germany

Method

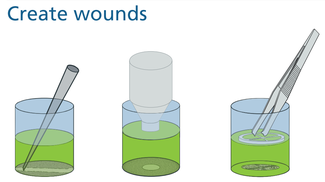
Count cells with Trypan Blue Application



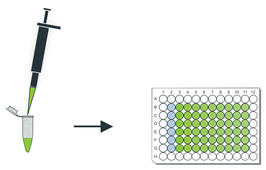
Seed cells



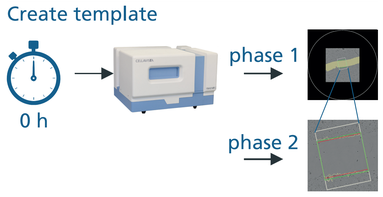
Create wounds



Treatment with Cytochalasin D



Create template



Continuous measurements of phase 2 and automatic data analysis

Introduction

Cell migration plays a crucial role in many physiological processes such as wound healing, tissue formation, and the immune response, but it is also pathologically relevant in cancer invasion and metastasis. A convenient method to analyze cell migration is a wound-healing assay, in which an artificial cell-free gap (wound) is created on a confluent monolayer of cells. Closure of the wound is monitored over time by microscopy. However, with conventional or time-lapse microscopes, only a few samples can be measured at a time. Therefore, we aimed to develop a wound healing assay with Cytochalasin D as an exemplary inhibitor in a high-throughput format with continuous measurement using the automated microscope CELLAVISTA®, our automation system, and image processing of YT®-Software (SYNENTEC).

Results

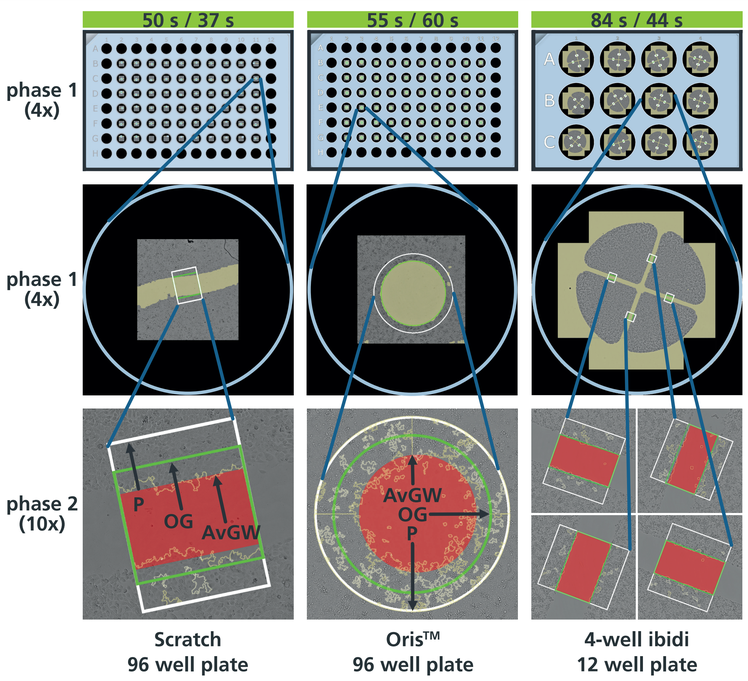


Fig. 1 The wound healing operator of YT®-Software consists of two phases and robustly detects wounds of different length, width, orientation and shape
Wounds in a monolayer of H6c7-Kras cells by manual scratching, Oris™ inserts (Platypus) or culture inserts 4-well (ibidi) were imaged by the 4x objective in phase 1. The image analysis algorithm of YT®-Software automatically detects the wounds (yellow), labels the detected area, and adds a defined periphery area. In phase 2, only this area is imaged using the 10x objective. The image processing application automatically analyzes the confluence in the original gap (OG, green) and the periphery (P, white) and determines wound closure as confluence and average gap width (AvGW, red). The imaging is very fast enabling a high throughput (exemplary scanning times for first phase / second phase).

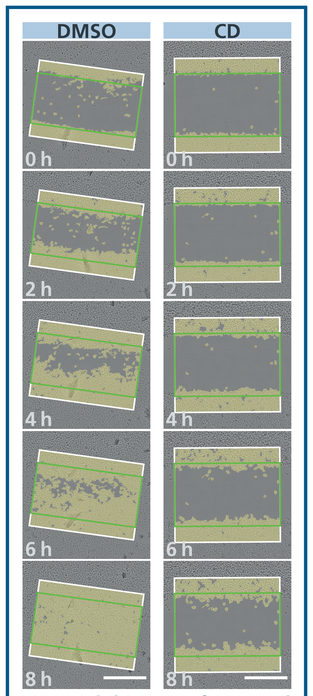


Fig. 2 Inhibition of wound closure by Cytochalasin D can automatically be monitored over time
Scratches were treated with DMSO or Cytochalasin D (CD) and imaged every hour. Cell confluence (yellow area) was automatically analyzed by the application (CD: 40 ng/mL, scale bar: 500 µm).

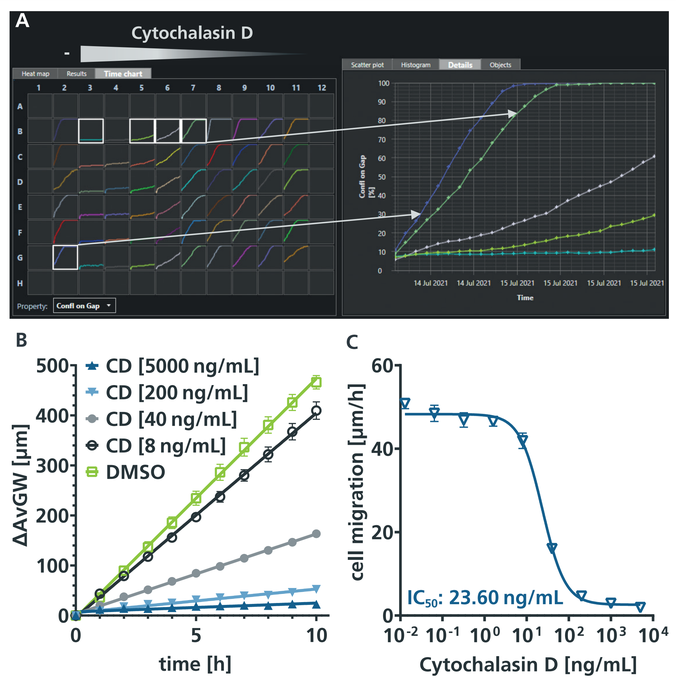


Fig. 3 YT®-Software automatically quantifies wound closure over time and depicted as time charts
H6c7-Kras cells were treated with different concentrations of the actin polymerization inhibitor Cytochalasin D (CD) or DMSO (solvent control). Wound closure was analyzed and depicted as confluence on gap (Confl on Gap) at multiple time points using the CELLAVISTA® and the wound healing application of YT®-Software (A). Data was exported and used to calculate the change in average gap width (ΔAvGW). This parameter, in contrast to the confluence on gap, displays wound closure regardless of original gap width, and the slopes represent cell migration (B). Cell migration was used for dose-response curve and IC_{50} determination (C).

Institut für Experimentelle Tumorforschung

Benefits of Wound Healing Assays in SYNENTEC's Automation System

- Automation-ready enabling a high throughput
- Continuous measurement over time
- Accurate and efficient image processing
- Flexible wound detection regardless of number, shape, orientation and size

Acknowledge
We thank the Institute for Experimental Tumor Research (Kiel) for a fruitful cooperation

Contact
Wiebe Schaefer and Reinhild Geisen
W.Schaefer@synentec.com, R.Geisen@synentec.com