Bioscience Solutions

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Simple and Easy Monitoring of Tube Formation and Migration Assays with the CytoSMART™ Live Cell Imaging System

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Watch the videos

1. Introduction

Movement of cells plays a critical role in the development of cancer. Analyzing the motility of cells *in vitro* is therefore important for many cancer researchers.

Live cell imaging, and in particular label-free live cell imaging, is well suited to capture dynamic processes in cell culture without potential side-effects of used markers or dyes on the cells. In this poster we show the suitability of the CytoSMART™ System for the analysis of different cancer-relevant assays.

2. The CytoSMART™ Lux 10X System

The CytoSMART™ Lux 10X System (Lonza) is an easy-to-use live cell monitoring system. The small footprint is ideal for placing into a standard cell culture incubator. It has been designed for applications, which require a larger field of view, such as cell culture documentation and migration assays. The field of view is 2.4 x 1.5 mm and the magnification is similar to that typically achieved with a 10x objective of a conventional microscope. The images and videos can be monitored anytime and anywhere, via smart phone, tablet or computer with the integrated cloud functionality.

Figure 1: (A) The CytoSMART™ Lux 10X System (B) Results can be monitored anytime and anywhere, via smart phone, tablet or computer

3. Tube Formation Assays

The formation of new blood vessels is required to ensure sufficient nutrient and oxygen supply and to allow solid tumors to grow beyond a certain size. This process can be mimicked in cell culture in so-called tube formation assays.

Human Umbilical Vein Endothelial Cells (HUVEC; Lonza, P/N: C2519A) were seeded on 150 µL Basement Membrane Extract (BME; Matrigel™, Corning, P/N 356237) in standard 48-well cell culture plates. The formation of endothelial tubes was monitored live in selected wells.

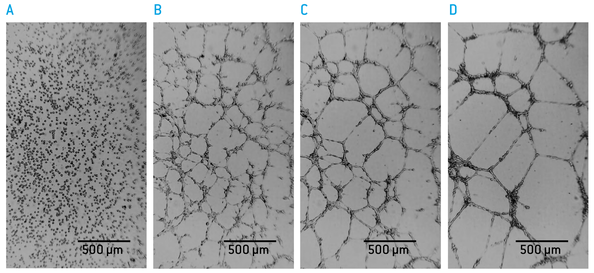

Figure 2: Tube Formation of HUVEC on BME (A) 0 hours, (B) 3 hours, (C) 6 hours and (D) 17 hours after seeding.

Cell aggregation into tube-like structures started immediately after seeding of cells and defined tube-like structures became visible after 4–6 hours. Tubes were stable for up to 24 hours. Understanding the kinetics of tube formation by live cell imaging allowed finding the optimal time-point for the quantitative analysis of the effect of Suramin (Sigma, P/N S2671) on tube formation.

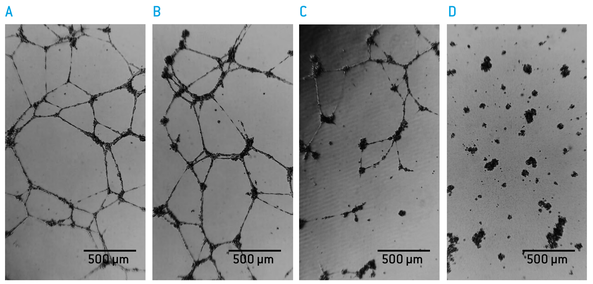

Figure 3: Tube Formation of HUVEC is inhibited by Suramin 18 hours after seeding (A) 0 µM Suramin, (B) 2.5 µM Suramin, (C) 7.5 µM Suramin and (D) 10 µM Suramin

Suramin inhibited the formation of tubular structures. For quantitative analysis, images were captured 18 hours after seeding in 3 different positions of each well by using the CytoSMART™ Lux 10X System like a standard cell culture microscope.

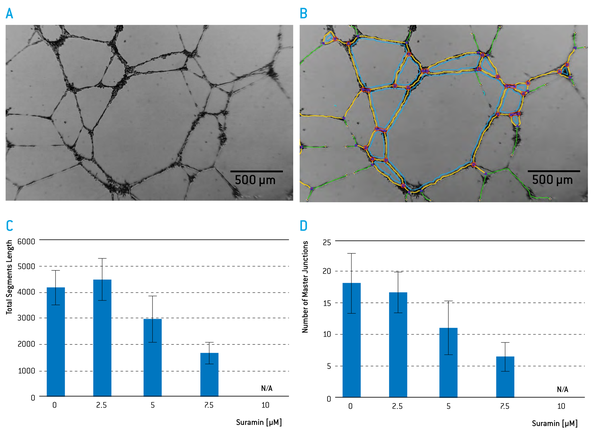

Figure 4: (A) Tube Formation of HUVEC on BME 18 hours after seeding. (B) Image Analysis with the Angiogenesis Analyzer Module of ImageJ. Tube segments are colored in yellow, green and blue and master junctions in pink. Impact of Suramin on (C) the total segment length and (D) the number of master junctions. (n = 6)

Using the Angiogenesis Analyzer Module of Image J (https://imagej.nih.gov/ij/) the number of master junctions and the total segment length per image were quantified. This was easily possible in all CytoSMART™ Images with the exception of images that were taken in the presence of 10 µM Suramin or higher. In these cases no clear distinction between actual tubular structures and debris could be made by the software.

4. 2D Cell Migration Assay

The migration of cancer cells is required for growth and metastasis of tumors. A basic test to measure the migration potential of cells is to monitor the closure of a so-called wound or scratch in a confluent monolayer of cancer cells.

The human colorectal carcinoma cell line HCT 116 (ATCC, P/N CCL-247) was seeded into 35mm micro-dishes containing a culture insert (ibidi, P/N 81176). After reaching confluence and removal of the culture insert a defined scratch is formed. Closure of the gap was monitored with the CytoSMART™ Lux 10X System.

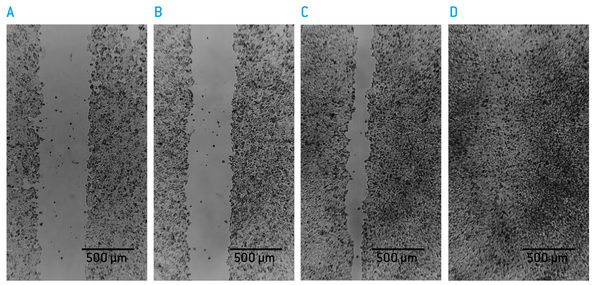

Figure 5: "Wound closure" of HCT 116 cells (A) 0 hours, (B) 15 hours, (C) 30 hours and (D) 40 hours after "wound creation"

Images were analyzed with the ImageJ MRI Wound Healing Tool or with the cell coverage measurement tool of the CytoSMART™ Lux 10X System in order to monitor the effect of DL-Sulforaphane (Sigma, P/N S4441) on gap closure.

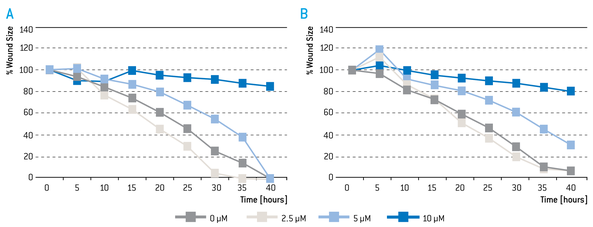

Figure 6: Kinetics of "wound closure" of HCT 116 cells exposed to different concentrations of Sulforaphane with the (A) ImageJ MRI Wound Healing Tool or the (B) cell coverage measurement tool of the CytoSMART™ Lux 10X System (n=1)

Gap closure could be quantitatively analyzed with both the ImageJ MRI Wound Healing Tool or the cell coverage measurement tool of the CytoSMART™ Lux 10X System. In samples treated with no or low amounts of Sulforaphane, gap closure is observed after ~35 hours. In samples treated with 5 µM Sulforaphane gap closure is delayed. In the presence of 10 µM Sulforaphane almost no reduction of gap width was observed.

The speed of gap closure varies among different cell types (data not shown). While some cell types close gaps within hours others take days to close a gap of similar size. Live cell imaging allows researchers to capture the kinetics of gap closure and to identify the optimal time point for quantitative image analysis.

5. 3D Invasion Assay

Migration of cancer cells *in vivo* often requires the movement through extracellular matrix. This process can be mimicked by embedding cancer cells into a three-dimensional (3D) matrix and monitoring their invasion into the 3D matrix.

The human, presumably glioblastoma cell line U-87 MG (ATCC, P/N HTB-14) was plated into round-well ultra-low attachment plates (Corning, P/N: 7007). After 3 days spheroids have formed and were overlaid with BME cell invasion matrix (Trevigen, PN 3500-096-K). Invasion of cells into the surrounding matrix was monitored with the CytoSMART™ Lux 10X System under constant temperature and CO_2 levels and without agitating the cultures. The migration properties of individual cells could be observed in real-time.

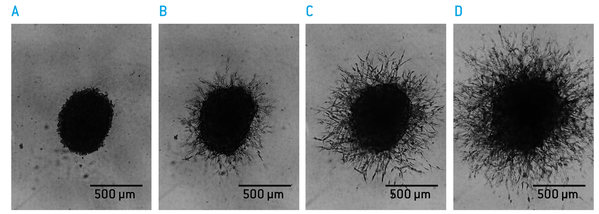

Figure 7: Sprouting of U-87 MG from a spheroid structure into a 3D matrix. (A) day 0, (B) day 1, (C) day 2 and (D) day 3.

The effect of DL-Sulforaphane on U-87 MG sprouting was quantified using Image J.

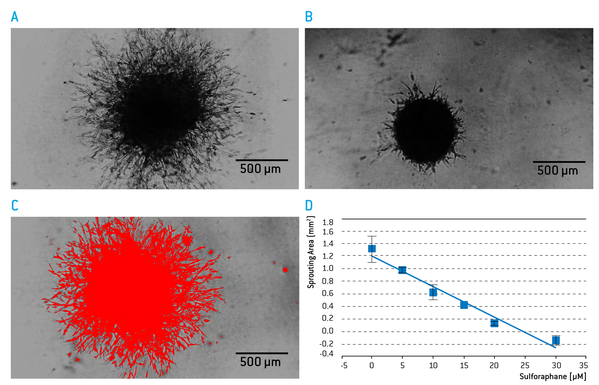

Figure 8: Sprouting of U-87 MG from a spheroid structure into a 3D matrix after 3 days of culture in the (A) absence or (B) presence of 30 µM Sulforaphane. (C) Quantification of sprouting with ImageJ. (D) Impact of Sulforaphane on sprouting (normalized to the size of spheroids on day 3 cultured in the absence of Sulforaphane and invasion matrix; n=2)

6. Conclusions

The CytoSMART™ System is an easy-to-use, live cell imaging system suitable for analysis of different cancer-relevant assays. Individual cells can be recognized in the resulting images. Therefore they can be quantified using software tools like ImageJ or the CytoSMART™ Analysis Software.