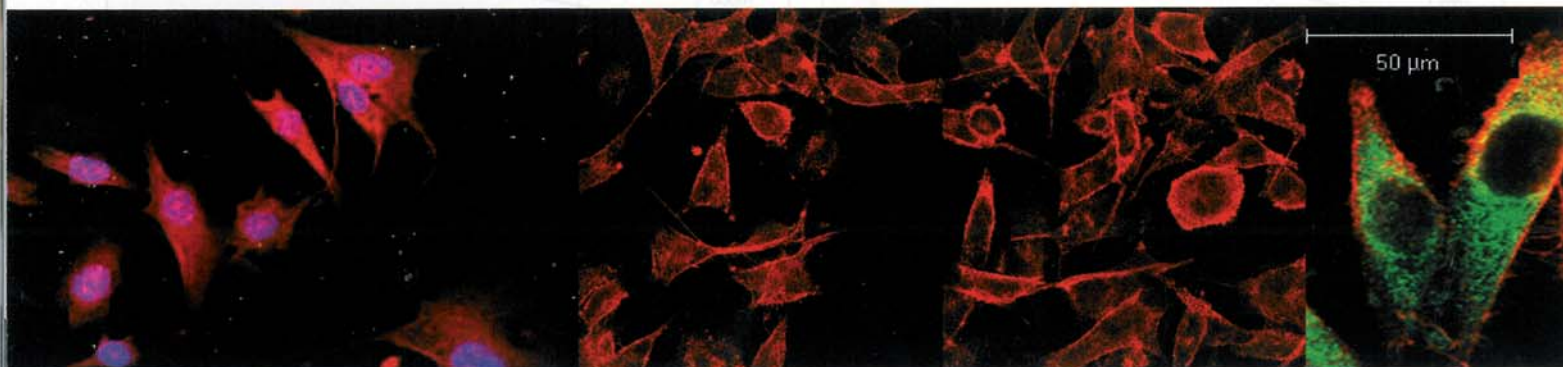


Migration Assay for High Throughput Screening (HTS)



Cell migration is a critical and central process in the development and maintenance of living organisms. Cells in the body will often move in a particular direction to a specific location to complete their functions. Migration is a cyclical process in which a cell extends protrusions at its front and retracts its trailing end. Cells in animal tissues mainly migrate in response to specific external signals.

The process of cell migration is important in such things as embryonic development, wound repair, differentiation and immune responses. Cell migration contributes to pathologies including vascular disease, chronic inflammatory diseases and tumor formation. Therefore, the search for components promoting (wound healing) or inhibiting (tumor formation) cell migration are therapeutically promising. We have established a novel migration assay for standardized and automated analysis in a highthroughput screening assay using the SpectraMax Paradigm Multimode Detection Platform from Molecular Devices. This assay offers an easier and more reproducible alternative to the scratch assay or other 2D wound closure assays.

Assay Description

The Oris Cell Migration Assay (non-coated) (Platypus Technologies, Madison, WI) utilizes cell seeding stoppers to create a pristine, 2 mm diameter detection zone in the center of each well. Human melanoma cells (50,000 per well) were dispensed into each well of a 96-well microplate populated with the cell seeding stoppers. After incubation over night the cells adhered to the surface around the stopper tips. The stoppers were removed and cells were either non-treated or treated with soluble chemokine CXCL9 in a concentration dependent manner (pos. contr.), with 100 ng/ml of different bioactive substances or with latrunculin (1 µg) as a negative control. Migration of

the melanoma cells proceeded for 16 hrs, cells stained with CellTracker Green (Invitrogen) and the Oris Detection Mask was applied to the bottom of the microplate (fig. 1). Cells that have migrated into the detection zone are imaged with the confocal microscope (Leica TCS SP2) and results confirmed quantitatively using the SpectraMax Paradigm Detection Platform (fig.2).

Results

To study and evaluate the migratory effect of novel isolated bioactive substances we used human melanoma cells and treated them with different potential migratory stimulating and inhibiting agents (chemokine CXCL9, latrunculin and bioactive substances). We assessed the degree of migration into the detection zone by measuring the fluorescence intensity after 16 hrs (end point measurement). Figure 3 summarizes the compound screening results for substances influencing the migratory behavior of melanoma cells. CXCL9 shows a dose dependent increase of fluorescence (migrated cells) in

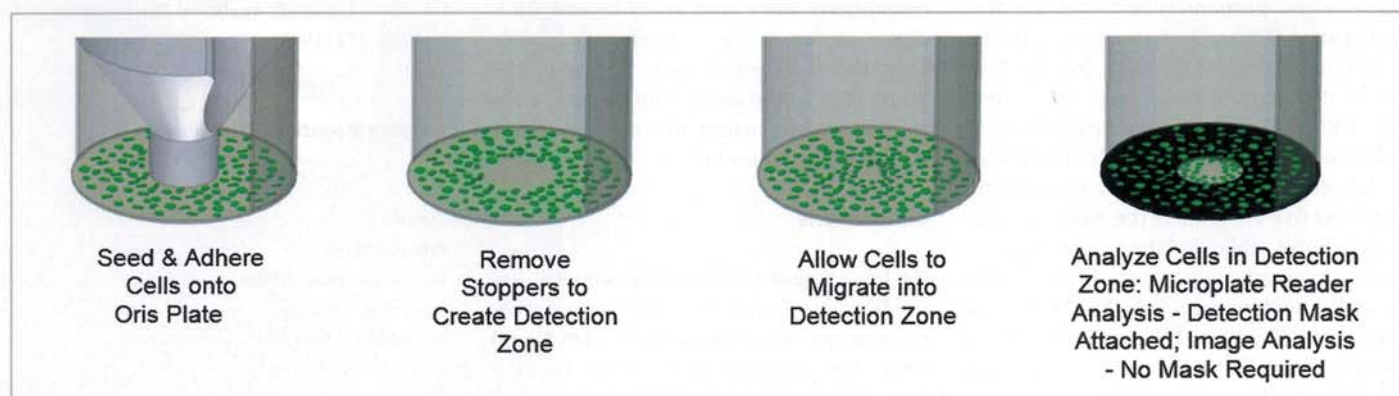


Fig. 1: Oris Cell Migration assay principle



Fig. 2: Paradigm Detection Platform

the detection zone whereas latrunculin indicates no effect. Although many bioactive substances display no or only a low migratory potential, substance 12 and 15 promote the migration of melanoma cells into the detection zone. Fold induction levels were calculated by dividing the fluorescence units (RFU) of each well to the mean of the RLU of the negative control. Mean and standard deviation of each sample was calculated.

Conclusion

This experimental system demonstrates a useful tool to measure cell migration for an HTS application. This study combines the Oris Cell Migration assay with

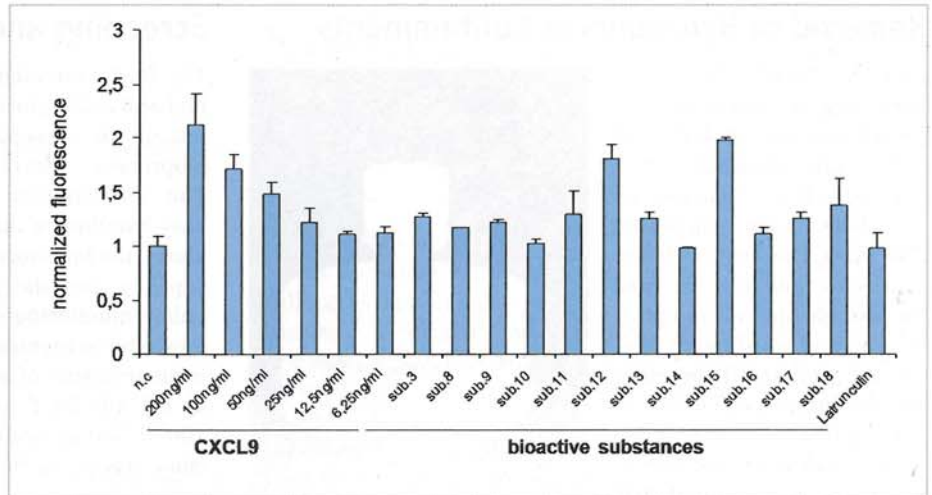


Fig. 3: Screening for potential stimulants and inhibitors of cell migration. The degree of melanoma cell migration was measured by reading the fluorescence intensity of the Oris Cell Migration Assay (with the detection mask attached to the bottom of the plate) using the SpectraMax Paradigm Detection Platform. Assay was performed in triplicate.

the SpectraMax Paradigm Detection Platform to quantify the degree of cell migration based on fluorescence intensity. This system is able to distinguish between the effects of various stimulants and inhibitors of cell migration.

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