

A Robust 384-Well Cell Migration Assay for High Content Analysis of Cells Treated with Anti-Cancer Therapeutics

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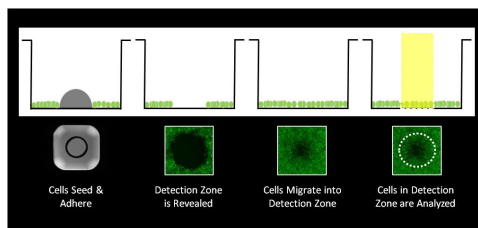
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Abstract

Platypus Technologies has developed a first-in-class 384-well cell migration assay to enable high throughput screening (HTS) of anti-cancer compounds and wound healing agents on adherent tumor and endothelial cell lines. The assay is completely automatable with liquid handling systems and high content analysis (HCA) instrumentation. This assay format utilizes a centrally located self-dissolving, non-toxic biocompatible gel (BCG) to form a uniformly sized, cell-free detection zone on tissue culture treated or collagen I coated cell culture surfaces. Cells are seeded into 384-well plates and pattern in an annular monolayer surrounding the BCG. Once the BCG dissolves, cells can migrate into the detection zone previously occupied by the BCG. Dose-dependent inhibition of migration was observed using the actin polymerization inhibitors Cytochalasin D and Latrunculin A, the kinase inhibitors Dasatinib and Sorafenib on HT-1080, and human umbilical vein endothelial cells (HUVECs), and the phosphatase inhibitors NSC95397 and BCI on MDA-MB231 human breast cancer cells. While migration of cells was faster on collagen coated surfaces than on tissue culture treated surfaces, the IC_{50} values obtained for the compounds and rank order of potency were similar on both surfaces. This assay format allows an unobstructed view of cell motility throughout the duration of the experiment. Cells may be fixed and treated with multiple stains, including Hoechst or DAPI to visualize nuclei and TRITC-phalloidin to observe F-actin, to enable flexible data capture by either enumerating migrating cells or by calculating the area of closure within the detection zone. The 384-well assay was successfully automated using a Biomek 2000 robotic liquid handler and a Cellomics ArrayScan II high content imaging (HCI) instrument.

Assay Schematic



Differential Data Analysis

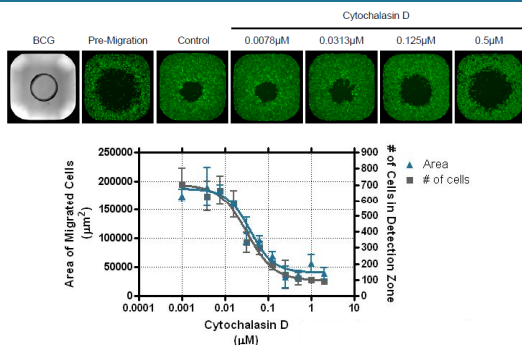


Figure 1. Effect of Cytochalasin D on HUVEC Migration. (A) Representative images of TRITC-phalloidin stained HUVEC cells in the presence and absence of Cytochalasin D (at indicated concentrations in μM) after 16 hr of migration as captured by a Zeiss Axiovert inverted microscope (pseudocolored green). (B) Dose-response curves as calculated by analyzing detection zones for area of migrated cells or number of cells using an Acumen *X3 scanning laser microplate cytometer (TTP LabTech). Data represents the average of 4 replicates/treatment condition \pm SD.

The calculated IC_{50} values for area and cell number determinations were 0.038 and 0.032 μM , respectively.

Image Acquisition Strategies & Multiparametric Analysis of Compound Effects

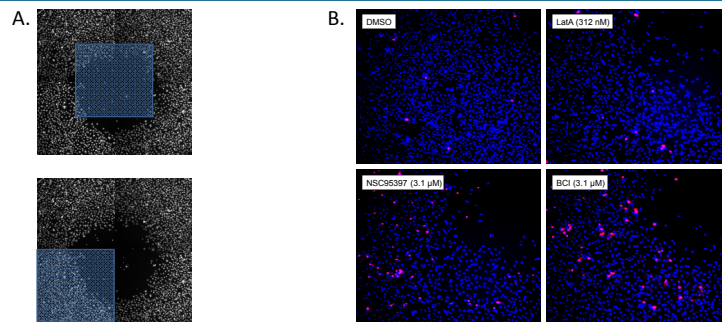


Figure 2. Strategies for HCA Image Acquisition. (A) Image Acquisition and Analysis of Cell Migration on Oris™ Pro 384 well Plates. Composite panels show Hoechst channel images of MDA-MB-231 cells acquired on a Cellomics ArrayScan II high content reader (ThermoFisher) using a 5X objective. **Top panel**, a single image taken in the center of the well captures cells that have migrated into the detection zone. **Bottom panel**, a single imaging field positioned at the edge of the detection zone yields sufficient numbers of cells for analysis of toxicity, morphology, and markers of compound activity. (B) Evaluation of Cytotoxicity of Compounds on MDA-MB-231 Cells at Corresponding IC_{50} Values for Cell Migration. MDA-MB-231 cells (8,000/well in 15 μL) were seeded in Oris™ Pro 384 collagen I-coated plates. After a 2 h incubation to allow for attachment, seeding media was removed and cells were treated with ten point, two-fold dilution gradients of the cytoskeleton-perturbing agents Cytochalasin D and Latrunculin A, or the dual specificity phosphatase inhibitors, NSC95397 and BCI. 48 h after treatment, cells were stained with 2.5 $\mu\text{g}/\text{mL}$ Hoechst 33342 and 1 $\mu\text{g}/\text{mL}$ propidium iodide to visualize cell monolayers and the proportion of cells with compromised membranes.

Cells treated with vehicle (0.5% DMSO) migrated in to the exclusion zone (shown in the upper right hand corner). Propidium iodide (PI) staining of live cells treated with DMSO shows low numbers of PI-positive cells (pink), documenting lack of cytotoxicity for both vehicle and the biocompatible gel. Latrunculin A inhibited cell migration without altering cell permeability. In contrast, NSC95397 and BCI showed increased numbers of PI positive cells, suggesting cellular toxicity may contribute to their anti-migratory effects.

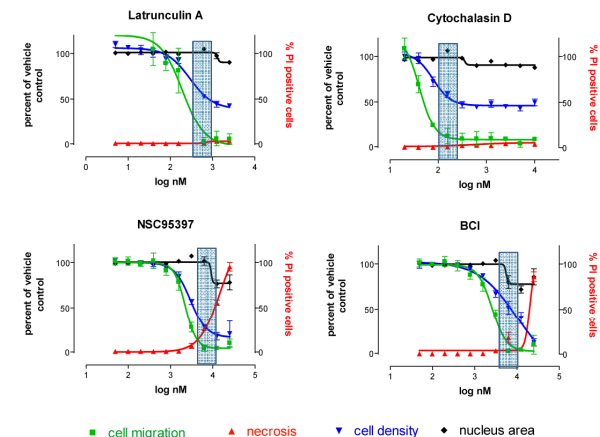


Figure 3. Multiparametric Quantification of Cell Migration and Toxicity. ArrayScan images of MDA-MB-231 cells stained with Hoechst 33342 and PI were analyzed with the Target Activation Bioapplication. Graphs show inhibition of cell migration (green), percentage of PI positive cells (red), cell density (blue), and changes in nuclear morphology (black). Data represent the average \pm SEM of 4 replicates for each concentration of compound. IC_{50} values for cell migration were calculated to be 40 nM for Cytochalasin D, 195 nM for Latrunculin A, 2.5 μM for NSC95397 and 2.6 μM for BCI. Concentrations of cytoskeletal perturbing agents that fully inhibited cell migration, as highlighted in the shaded rectangles, caused a modest change in cell density, but did not affect cell membrane permeability or nuclear morphology, indicating that cell migration was inhibited in the absence of gross toxicity. The window for anti-migratory activity vs. cell loss was greater than 10-fold for both agents. In contrast, NSC95397 and BCI caused changes in cell membrane permeability, profound cell loss, and nuclear condensation that occurred at 2-3 x the IC_{50} for migration inhibition.

Multiparameter analysis of cell migration and toxicity documents different activity profiles for the two different classes of anti-migratory agents. The data suggest the two phosphatase inhibitors have a dual mode of action that includes inhibition of both cell migration and proliferation.

Efficacy of Anti-Cancer Therapeutics on Cell Migration

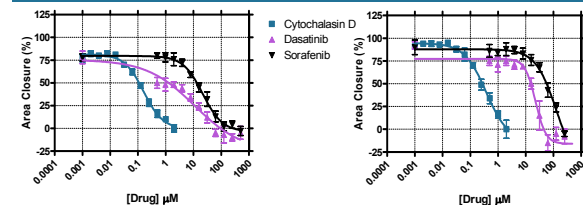
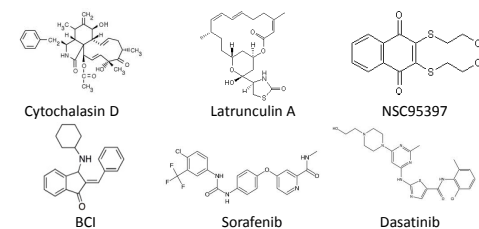


Figure 4. Effects of Cytochalasin D, Dasatinib and Sorafenib on HUVEC (Left panel) and HT-1080 (Right panel) Cell Migration. HUVEC (4,500 cells/20 μL) or HT-1080 cells (5,000 cells/20 μL) were seeded into Oris™ Pro 384 Collagen I coated assay plates. After 1 hr, compounds at indicated concentrations or 0.1% DMSO vehicle were added in additional 20 μL aliquots of serum-containing growth medium. After 16 hr of migration, the cells were fixed and stained with TRITC-phalloidin. Images were captured by a Zeiss Axiovert inverted microscope and percent area closure of migrated cells was calculated by analyzing detection zones using ImageJ software (NIH). Data represents the average of 4 replicates per treatment condition \pm SD.

The calculated IC_{50} values for Cytochalasin D, Dasatinib and Sorafenib were 0.161, 10.9 and 21.5 μM for HUVEC and 0.377, 22.4 and 129.8 μM for HT-1080 cells, respectively.

Compound Structures



Conclusions

- The Oris™ Pro 384 Cell Migration Assay is a robust, easily automatable assay that provides complete visual access to the cells and is suitable for high throughput screening (HTS) and high content analysis (HCA).
- The Oris™ Pro 384 Cell Migration Assay is suitable for use in screening the effects of anti-cancer compounds on multiple cell lines and on multiple assay well surfaces.
- The Oris™ Pro 384 Cell Migration Assay allows for multiparametric analysis of compound effects such as changes to membrane permeability, cell density and nuclear morphology.
- Data may be captured from the Oris™ Pro 384 Cell Migration Assay using scanning laser microplate cytometers or high content image analyzers and can be analyzed as either percent area closure or numbers of cells in the detection zone.
- Cytochalasin D, Dasatinib and Sorafenib exhibit the same rank-order of potency on HUVEC and HT-1080 cells.

Acknowledgements

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