A 3-Dimensional Cell Invasion Assay Compatible with High Content Screening

Jennifer A. Fronczak*, Matthew S. Gajeski*, Erica L. Beckman*, Andreas Vogt§, and Gopal B. Krishnan*

§University of Pittsburgh Drug Discovery Institute, Pittsburgh, PA 15260 USA

* Platypus Technologies, LLC., Madison, WI 53711 USA

www.platypustech.com

Abstract

The transition from non-invasive phenotype to invasive phenotype of tumor cells marks the switch from a benign tumor that can be successfully treated via surgery to a more malignant form of cancer. Understanding the mechanisms underlying this hallmark event, which enables tumor cells to invade through extracellular matrix, is critical for discovering pathways and new targets to develop anti-metastatic strategies. Previously, we developed a cell based assay for the study of cell migration in 96-well plates. A self-dissolving biocompatible gel (BCG) is utilized to form uniformly sized, cell-free detection zones in collagen I coated 96-well plates. When cells are seeded into these wells, they pattern in an annular monolayer surrounding the BCG. Once the BCG dissolves, an overlay of collagen I is applied to the assay wells, following which cells can invade in 3-dimensions into the detection zone previously occupied by the BCG. This "Oris™ Pro" cell invasion assay presents a straightforward, accessible and quantifiable cell method to study cell invasion, and can be used for example to evaluate candidate drugs targeting tumor invasion. Microscopic visual assessment of cell invasion in the presence of inhibitors is made by use of cell stains at multiple time-points. In this study we demonstrate the use of this assay platform for robust and reproducible analysis of HT-1080 cell invasion in 3-D. The presentation discusses optimization of cell based assay parameters such as (i) density of collagen overlay and time; (ii) monitoring of cell invasion in the presence of inhibitors: mitogen activated protein kinase inhibitor UO126 and actin polymerization inhibitor Cytochalasin D, and (iii) the qualitative and quantitative readouts that can be obtained using the 3-dimensional cell invasion assay.

Assay Overview

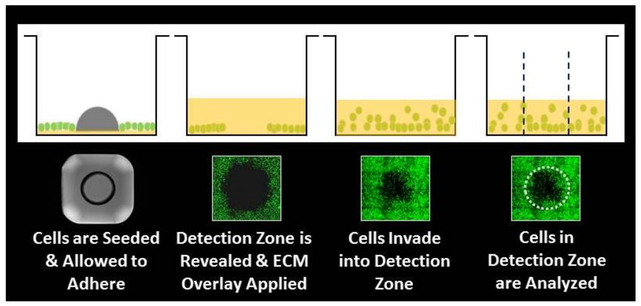


Figure 1: Oris™ Pro 96-well Cell Invasion Assay.
Cells are seeded and allowed to adhere in an annular monolayer surrounding the Oris™ Pro BCG. Collagen I overlay matrix is added. Cells invade into the Detection Zone (DZ). Invasion is imaged and analyzed via fluorescence microscopy or High Content Imagers.

Invasion Assay Workflow

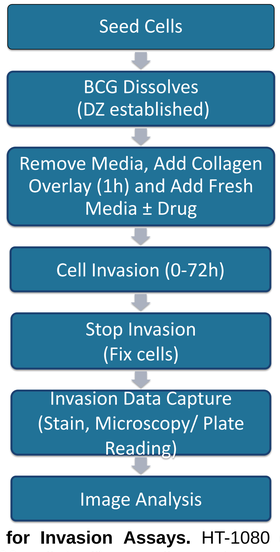


Figure 2: Workflow for Invasion Assays. HT-1080 cells were seeded at optimal density (30,000 cells/well) and allowed to adhere before removal of media. 40 µL Rat Tail Collagen Type I was overlaid at 2 or 3 mg/mL, followed by addition of fresh media ± drug. Cell invasion was assayed at multiple intervals by fixing cells with 0.25% gluteraldehyde, staining with DAPI, and imaging using a Zeiss Observer fluorescence microscope.

Imaging Invasion and Analysis

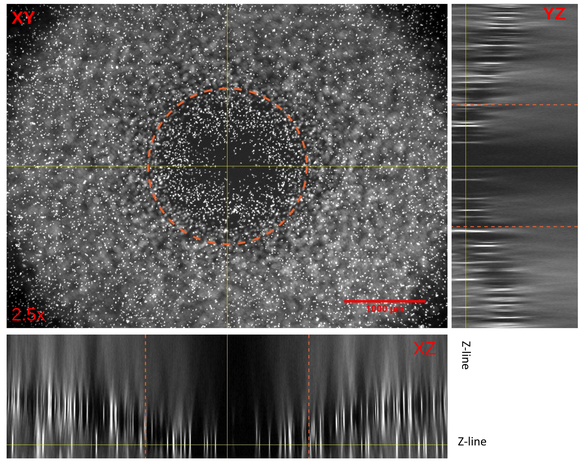
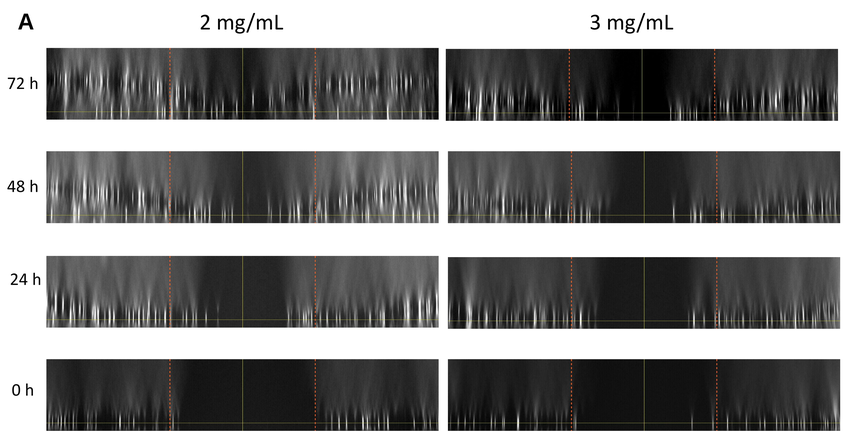


Figure 3: Image analysis using ImageJ to generate orthogonal view images. Z-stack images of cells were analyzed using ImageJ (NIH) software. Shown above is a representative image taken at 2.5x magnification. Stacks of images were taken at optimal Nyquist rates of 21.39 µm and processed to obtain orthogonal views of the XZ plane (bottom panel) and YZ plane (right panel). The Z-lines were set at the zero plane for each well. Total height of Z-stacks are 1519 µm. The orange dashed lines superimposed onto all images, indicate the boundaries of the starting cell-free detection zones.

Effect of Collagen Overlay Density and Time Course of Oris™ Pro Invasion Assay



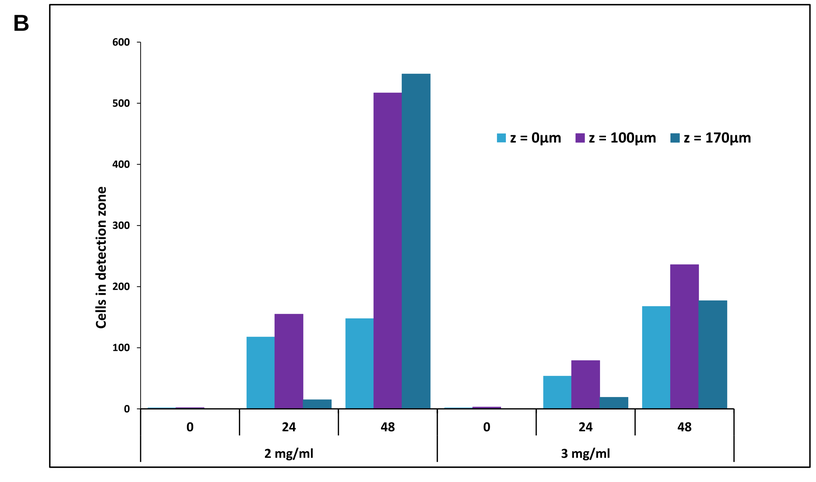
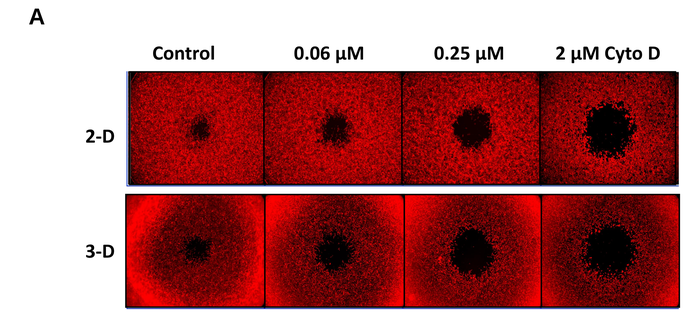


Figure 4: Decreasing the concentration of the collagen overlay facilitates invasion as a function of time in the cell invasion assay . Cell invasion assay was performed by varying matrix overlay with 2 or 3 mg/mL collagen and assayed at 0, 24 and 48 h. The results demonstrate an increase in cell invasion as a function of time and lower levels of invasion in the higher concentration collagen matrices. (**A**) Orthogonal views demonstrate cell movement into the DZ (orange lines). At 48 h significant invasion into the Z-plane and DZ was observed with 3mg/mL allowing the least amount of cellular movement. **(B)** Quantification of invasion corroborates visual observation – increase in invasion over time and decrease with higher concentration of collagen matrices, at different Z heights (0 µm,100 µm,170 µm).

Inhibition of 2-D Migration and 3-D Invasion



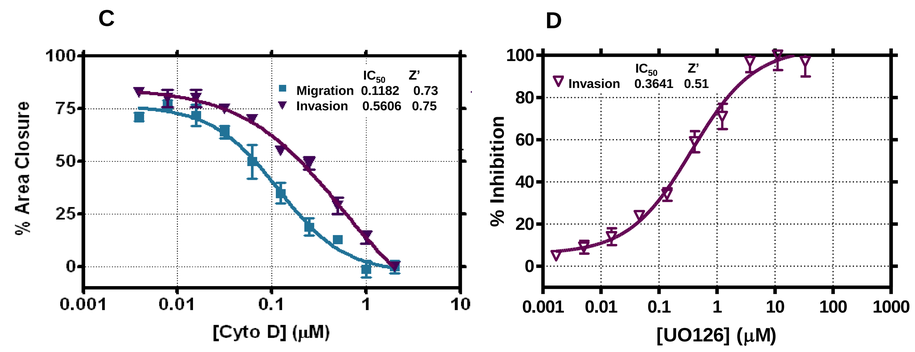


Figure 5: Inhibition of 2-D migration and 3-D invasion with Cytochalasin D and UO126: Cell migration and invasion were assayed at 21 and 45-48h respectively, with a 2 mg/mL Collagen I overlay for invasion plates alone, in the presence of inhibitors. The plates were imaged using fluorescence microscopy: **(A)** 2-D migration in control (0.1% DMSO) and indicated concentrations of Cytochalasin D **(B)** Orthogonal view of images using ImageJ of 3-D invasion plates demonstrates invasion in control and inhibitor treated plates. Representative images taken at 2.5x magnification and 877 µm in Z-height. **(C)** Results demonstrate inhibition of both 2-D and 3-D motility by Cytochalasin D. However dose required is higher for invasion (purple line) as indicated by higher IC50 value (0.5606 µM) compared to migration (blue line) (0.1182 µM). **(D)** Cell Invasion in control (0.1% DMSO) and in presence of invasion inhibitor UO126 demonstrates dose dependent inhibition with an IC50 value of 0.3641 µM.

Summary and Conclusions

Assay conditions for the Oris™ Pro 96-well Cell Invasion Assay can be optimized by varying collagen concentration & time-point.

Images of invading cells in the Z-plane can be captured by fluorescence microscopy and analyzed using ImageJ (NIH).

Orthogonal view images demonstrate the extent of cell invasion into the Z-plane.

The degree of 3-D invasion is dependent on concentration of collagen in the overlay and is time dependent.

Cell motility is inhibited using Cytochalasin D. The IC_{50} values are different for 2-D migration versus 3-D invasion. Cell invasion is inhibited by UO126 in a dose dependent manner.

The Oris™ Pro Cell Invasion Assay is versatile, allowing convenient 3-D cell invasion analysis with unrestricted access, measurement of cellular invasion in the Z-plane, and compatibility with automation and HCS.

Acknowledgements

This work was partially supported by grant 2R44GM090386-02 from the National Institute of General Medical Sciences (NIH)