Analysis of Multiple Cell Lines and Varying Collagen Concentrations in a 3-Dimensional Cell Invasion Assay

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Abstract

The transition from a non-invasive phenotype to an invasive phenotype marks the switch from a benign tumor to a more malignant form of cancer in which tumor cells invade through the extracellular matrix. Understanding the mechanisms underlying this hallmark event is critical for discovering metastatic pathways and new targets for anti-metastatic strategies. Platypus Technologies developed the Oris™ Pro Cell Migration Assay for the study of cell migration in 2-D. The assay uses a nontoxic biocompatible gel (BCG) to generate cell-free exclusion zones that are centrally positioned in 96- and 384-w plates. This assay has been successfully used to screen compound libraries of cancer specific and pharmacologically active drugs. The Oris™ Pro Cell Migration Assay has been developed to be a versatile and accessible 3-D cell invasion assay. The self-dissolving BCG is utilized to form uniformly sized cell-free detection zones in wells of collagen I coated multiwell plates. Once the BCG dissolves, following the addition of cells with media, an overlay of collagen is added to the wells into which cells can then invade in 3-dimensions into the Detection Zone. Microscopic assessment of cell invasion can be monitored in the presence of potential inhibitors throughout the duration of the experiment. The **Oris™ Pro Cell Invasion Assay** provides an automation compatible model, which allows for both an increased capacity and decreased hands-on time for screening assays, to evaluate candidate drugs targeting tumor invasion. This is an attractive option for High Throughput Screening and High Content Analysis of modulators of cell motility. The current study demonstrates a method of measuring invasion of HT-1080 and MDA-MB-231 cell movement in 3-D using ImageJ (NIH) software. The presentation discusses optimization of cell based assay parameters and qualitative measurement of cell invasion in 3-D using an orthogonal view generated by ImageJ software.

Assay Schematic

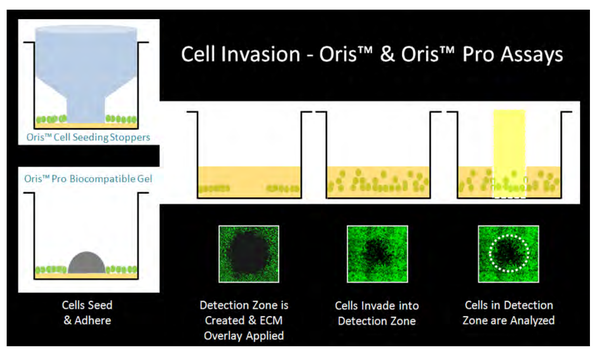

Figure 1: Oris™ and Oris™ Pro Cell Invasion Assay.
Cells are seeded and allowed to adhere in an annular monolayer surrounding the Oris™ Cell Seeding Stoppers or Oris™ Pro Biocompatible Gel (BCG). The appropriate overlay matrix is added. Cells invade into the Detection Zone (DZ). Cells are imaged via fluorescence microscopy or High Content Imagers. Images are then analyzed for cell invasion as well as phenotypic changes.

Invasion Assay Workflow

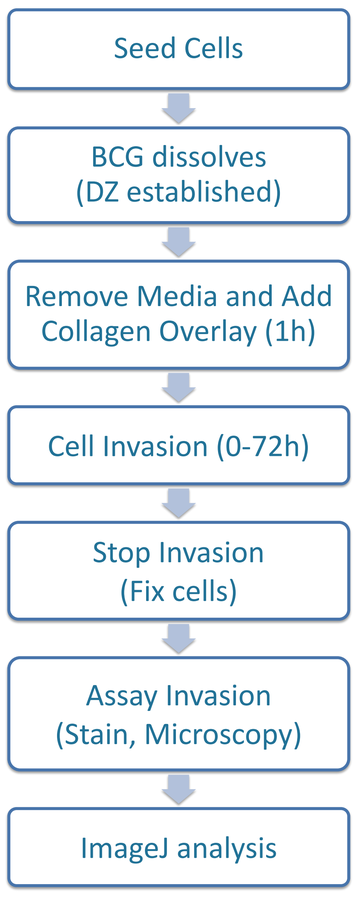

Figure 2: Workflow for Invasion Assays. Oris™ Pro 96 Cell Invasion Assay setup, microscopy, and image analysis. HT-1080 or MDA-MB-231 cells were seeded at optimal density (30,000 cells/well) and allowed to adhere before removal of media. 40 µL Rat Tail Collagen Type I was overlaid at 1, 2, or 3 mg/mL. 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

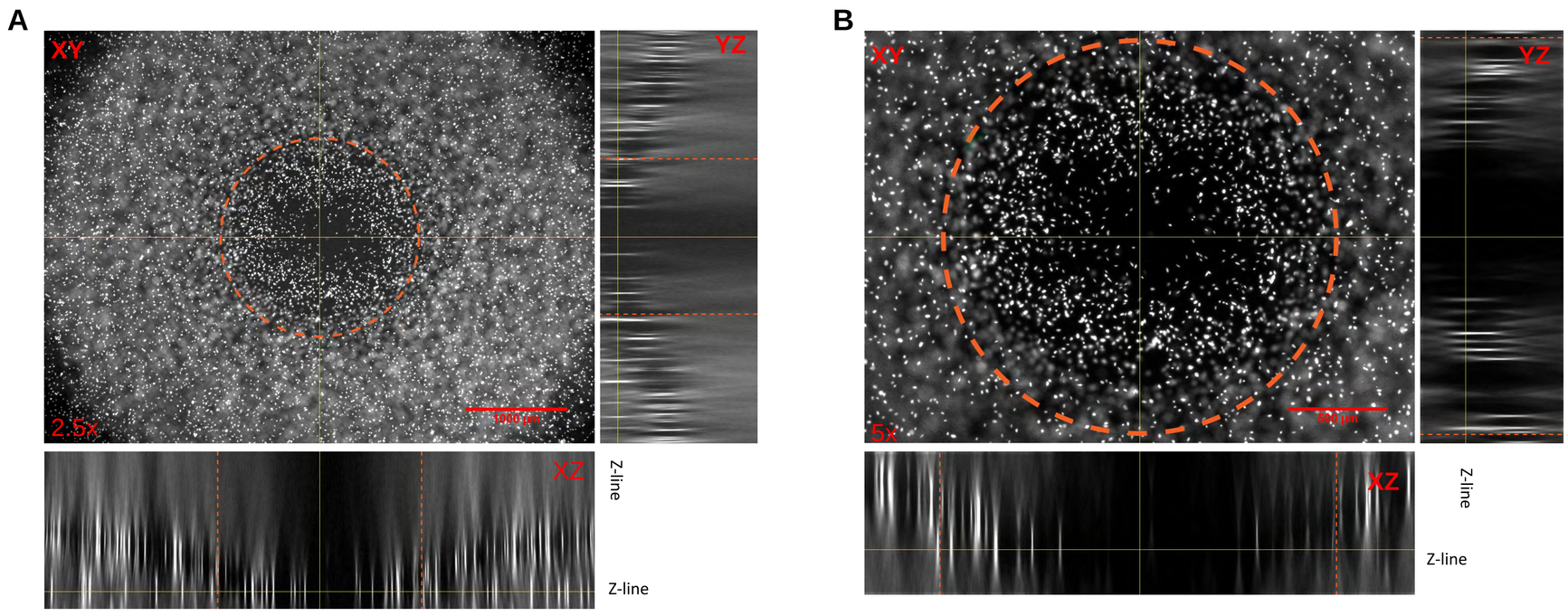

Figure 3: Image analysis using ImageJ to generate orthogonal view images. Z-stack images of HT-1080 cells were analyzed using ImageJ (NIH) software. Representative images shown above were taken at (A) 2.5x and (B) 5x magnification. Stacks of images were taken at optimal Nyquist rates (A) 21.39 µm and (B) 12.03 µm. Images were 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 (A) 1519 µm and (B) 1516 µm. An outline of the 2 mm diameter Detection Zone (orange dashed lines) serves as a reference point for cell invasion which has been superimposed onto all images accordingly.

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

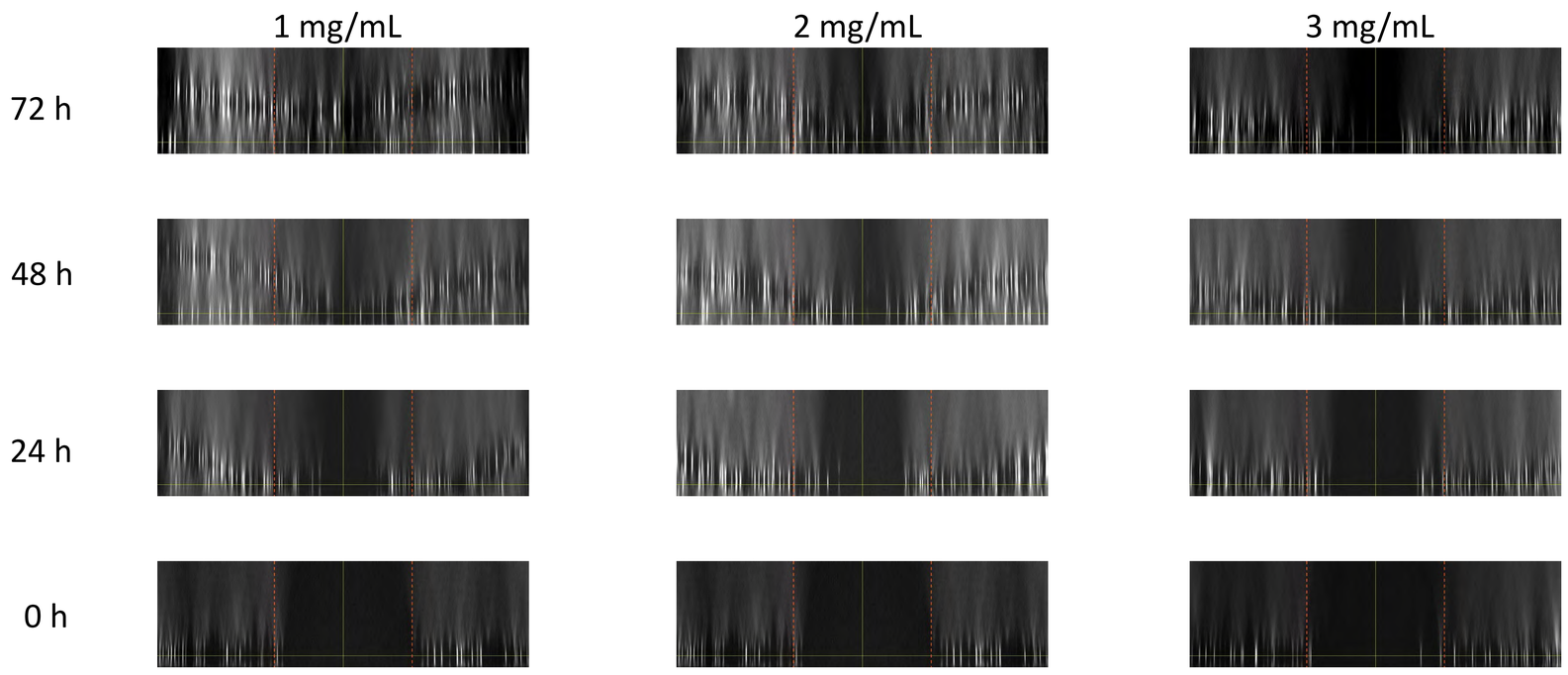

Figure 4: Effect of collagen overlay density as a function of time in cell invasion assay: Increasing the concentration of the collagen overlay restricts the invasion of HT-1080 cells. An Oris™ Invasion Assay was performed with HT-1080 cells overlaid with 1, 2, or 3 mg/mL collagen and assayed at 0, 24, 48, and 72 h. The results demonstrate an increase in cell invasion as a function of time. Lower levels of invasion were seen in the higher concentration collagen matrices. At 24 h, cell movement into the Detection Zone (dashed vertical lines) was observed. At 48 h and 72 h, significant invasion into the Z-plane and Detection Zone was observed with 3mg/mL allowing the least amount of cellular movement. Representative images shown were taken at 2.5x magnification (12 replicates per condition) and are 1519 µm in Z-height.

Extent of Invasion is Cell Line Specific

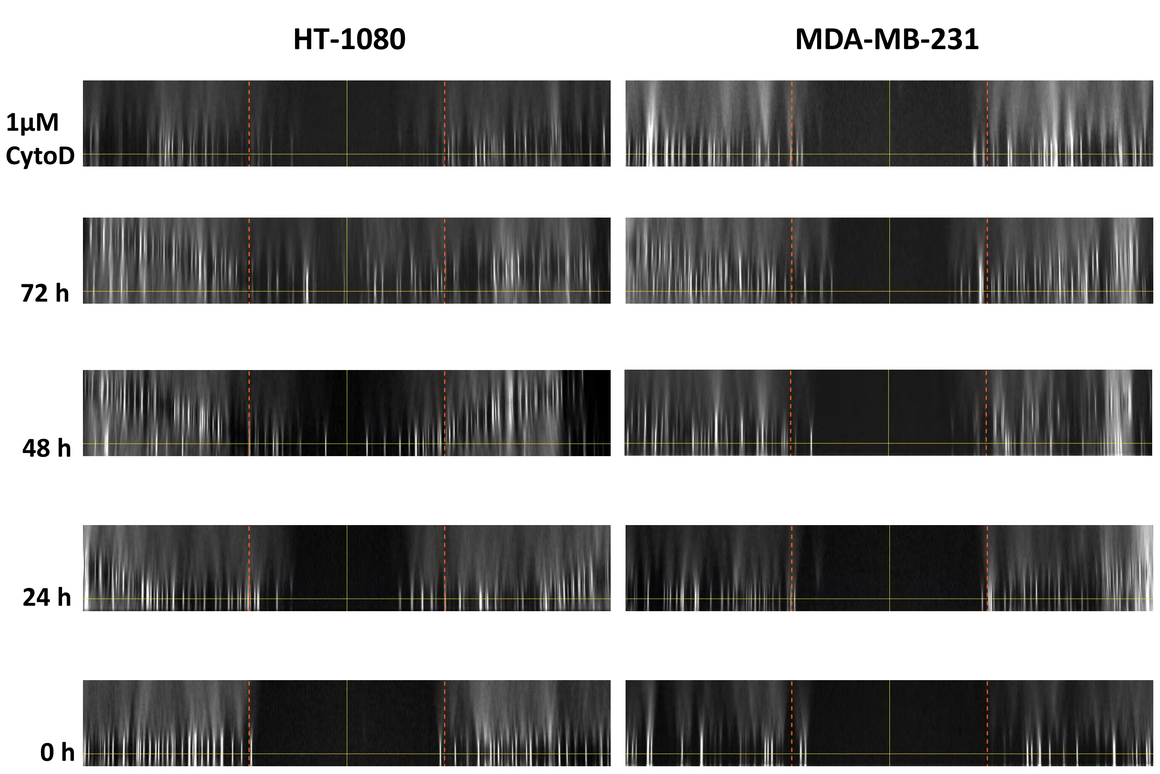

Figure 5: Invasion in HT-1080 and MDA-MB-231 cell-lines: HT-1080 and MDA-MB-231 cells were assayed at 30,000 cells/well with a 2 mg/mL Collagen I overlay. Invasion plates were fixed at 0, 24, 48, and 72 h respectively. Representative images shown above were taken at 2.5x magnification (12 replicates per condition) and are 877 µm in Z-height. The results indicate more invasion is seen with the HT-1080 cells with cells invading into the Detection Zone by 24 h and significant invasion in the Z direction by 48 h. MDA-MB-231 cells demonstrate some invasion in the Z direction by 72 h.

Summary and Conclusions

Assay conditions for the Oris™ Pro Cell Invasion Assay setup were successfully optimized.

Images of invading cells in the Z-plane were captured by fluorescence microscopy and analyzed using ImageJ software from NIH.

Orthogonal view images demonstrate the extent of invasion along the Z-plane under varying conditions of collagen overlay concentration and time.

Invasion is cell line specific and time dependant. In the Oris™ assay, HT-1080 cells are more invasive than MDA-MB-231 in equivalent conditions.

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

Acknowledgements

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