Comparison of the Oris™ and Oris™ Pro Cell Migration Assays

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Technical Memo

ORIS™ CELL MIGRATION ASSAYS

Summary

The first generation Oris™ Cell Migration Assay uses silicone stoppers to create cell exclusion zones in tissue culture wells. This assay requires the user to physically remove the stoppers following cell seeding in order to reveal the Detection Zone into which cells could migrate. The new Oris™ Pro Cell Migration Assay replaces the stoppers with a Biocompatible Gel (BCG) that provides a self-dissolving barrier to form the cell-free central Detection Zone. We demonstrate herein that the BCG does not affect cell viability or cytotoxicity of either HT-1080 cells or human umbilical vein endothelial cells (HUVECs). We also show that the amount of cell migration and effects of 3 different classes of inhibitors on these cell lines are substantially equivalent in the Oris™ and the Oris™ Pro Cell Migration Assays.

Introduction

Cell migration plays a critical role in many physiological and pathological processes, such as wound healing and cancer metastasis[1]. There is a significant effort to screen compound libraries for modulators that inhibit abnormal migration of cancer cells or promote migration of cells to stimulate wound healing in order to discover therapeutics for a diversity of pathological processes. Investigators are seeking improved assays that offer 1) the ability to view cells in real-time during experiments, a feature that is lacking in Boyden chamber based assays and 2) greater reproducibility than in scratch assays where wound sizes can be inconsistent. Platypus Technologies has developed a series of cell migration assays that address these required improvements based on the formation of central cell-free Detection Zones in assay wells. These assays are the Oris™ and Oris™ Pro Cell Migration Assays.

The Oris™ Pro Cell Migration Assay (Figure 1A) is a 96-well cell exclusion assay that may be useful for screening compound libraries. It is provided with spots of Biocompatible Gel (BCG) centrally deposited in each well to exclude cells from adhering in the centers of the wells. After cells are seeded and allowed to adhere, the BCG dissolves to reveal reproducible cell-free Detection Zones in the center of each well into which cells are permitted to migrate. The Oris™ Pro Cell Migration Assay offers unlimited access to test wells by automated liquid handling equipment to facilitate efficient delivery of cells, media and test compounds thereby decreasing hands-on time required by laboratory personnel. This assay format allows improved reproducibility and increased robustness over wound healing/scratch assays and is compatible with High Content Imaging (HCI) systems.

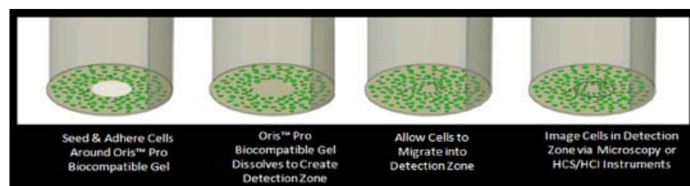


Figure 1A. Oris™ Pro Cell Migration Assay Schematic

In contrast, the previously established Oris™ Cell Migration Assay utilizes silicone Oris™ Cell Seeding Stoppers to form the Detection Zones (Figure 1B), which require manual cell seeding and must be manually removed to initiate the assay and thus may be more limited for high throughput screening.

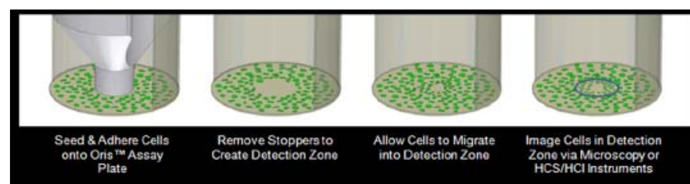


Figure 1B. Oris™ Cell Migration Assay Schematic

Materials & Methods

Cytotoxicity and Cell Viability: Cell type specific media (50 µL) was added to wells of Oris™ Pro Collagen I plates in the presence or absence of BCG and incubated for 4hr at 37°C/5% CO_2. Cells (2,500 in 50 µL media) were added to all test wells and incubated for 18 hr. The Vybrant™ Cytotoxicity Assay (Molecular Probes) was used to measure the amount of glucose 6-phosphate dehydrogenase (G6PD) released from damaged cells, and the CellTiter96® AQueous One Solution Cell Proliferation Assay (Promega) was used to measure metabolic function through reduction of formazan. Fluorescence and absorbance were measured, respectively, for these assays using a BioTek Synergy™ HT microplate reader.

Cell Migration: HT-1080 fibrosarcoma cells (30,000 cells/well) and human umbilical vein endothelial cells, HUVECs (25,000 cells/well) were seeded onto Collagen I coated plates in the Oris™ Pro and Oris™ Cell Migration Assays. After 1 hour stoppers were removed from the control and test wells of the Oris™ plates; the BCG self-dissolved in the wells of the Oris™ Pro plates. For pre-migration references, stoppers remained in a portion of wells of Oris™ plates until staining occurred whereas images were captured at 1 hr post-seeding for pre-migration wells of Oris™ Pro plates. Cells were incubated for 18 hr in the presence or absence of migration inhibitors, followed by fixation and staining with TRITC-phalloidin. Images were acquired using either a Zeiss Axiovert 200 inverted microscope equipped with a CCD camera or a BD Pathway™ 855 Bioimaging System. Cell migration was analyzed as the percent closure of the Detection Zone using ImageJ.

[1] Ridley et al., 2003. Science. 302(5651):1704-1709.

Results

A principal component of the Oris™ Pro Cell Migration Assay is the BCG that forms the cell exclusion zone in the assay wells. We conducted experiments to demonstrate that BCG is not cytotoxic and does not affect cell viability. Assessments of cytotoxicity and viability were performed on HT-1080 and HUVEC cells in the presence and absence of BCG by using the Vybrant™ Cytotoxicity Assay and CellTiter96® AQueous One Solution Cell Proliferation Assay, respectively. Results of cell health after 18 hr incubation in wells containing dissolved BCG are shown in Figure 2. Similar levels of G6PD released from damaged cells (Figure 2A) and levels of metabolic function (Figure 2B) measured for each cell type in the presence and absence of dissolved BCG indicate that the BCG provided in the Oris™ Pro Collagen I Assay is non-toxic to HT-1080 and HUVECs.

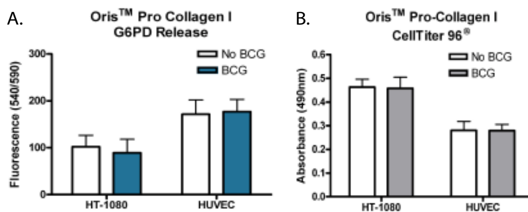
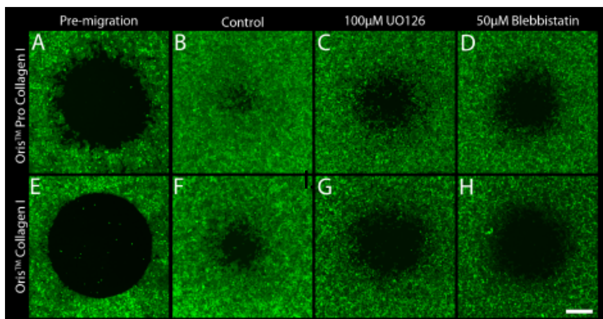


Figure 2. Effect of BCG on HT-1080 and HUVEC Cytotoxicity and Cell Viability using G6PD Release (A) and Cell Titer 96® (B). Data represent the average fluorescence and absorbance values ± SD for a minimum of 14 wells per condition. Results indicate no substantial effects of BCG on cytotoxicity or cell viability.

We next examined the ability of cells to migrate and to respond to inhibitors in both the Oris™ Pro and Oris Cell Migration Assay formats. Direct comparisons were performed for HT-1080 cell migration in the presence and absence of a MEK inhibitor (UO126) and a Myosin II inhibitor (Blebbistatin) on Collagen I coated plates in the Oris™ Pro and Oris™ Assays. Migration of HT-1080 cells after 18hr was identical in both the Oris™ Pro (Figures 3A and 3B) and Oris™ Assays (Figures 3E and 3F) as measured by percent area closure of the Detection Zones. The inhibitory effects of 100 µM UO126 and 50 µM Blebbistatin on HT-1080 cell migration were also equivalent (Figures 3C, 3D, 3G and 3H) with no statistically significant differences between assay formats (Figure 3J).



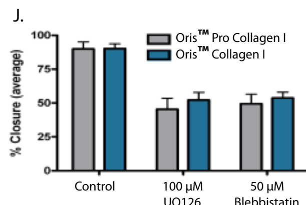


Figure 3. Effects of the MEK Inhibitor UO126 & the Myosin II Inhibitor Blebbistatin on HT-1080 cell migration in the Oris™ Pro and Oris™ Collagen I Assays. Representative images of cells labeled with TRITC-phalloidin (pseudo-colored green) in the Oris™Pro (A-D) and Oris™ (E-H) assays. Images of pre-migration (A and E), 18hr migration controls (B and F), and cells treated with 100 µM UO126 (C and G) and 50 µM Blebbistatin (D and H) are shown. Scale bar=500 µm. Data are presented as the average percent closure of the Detection Zones ± SD from 16 wells for each condition (J). Results indicate substantially similar amounts of migration and responses to inhibitors between assay formats.

Additionally, a comparative dose response for the actin polymerization inhibitor, Cytochalasin D, was performed with HUVECs in the Oris™ Pro and Oris™ Assays. Cell migration was assessed on Collagen I coated plates in the presence of 0.0078 µM to 2 µM Cytochalasin D and was measured by calculating the percent closure of the Detection Zones after 18hr using the Oris™ Pro (Figure 4A) and Oris™ (Figure 4B) Cell Migration Assays. HUVEC migration in the presence of Cytochalasin D yielded super-imposable dose response curves and overlapping IC_{50} values (Figure 4C) in the two assay formats.

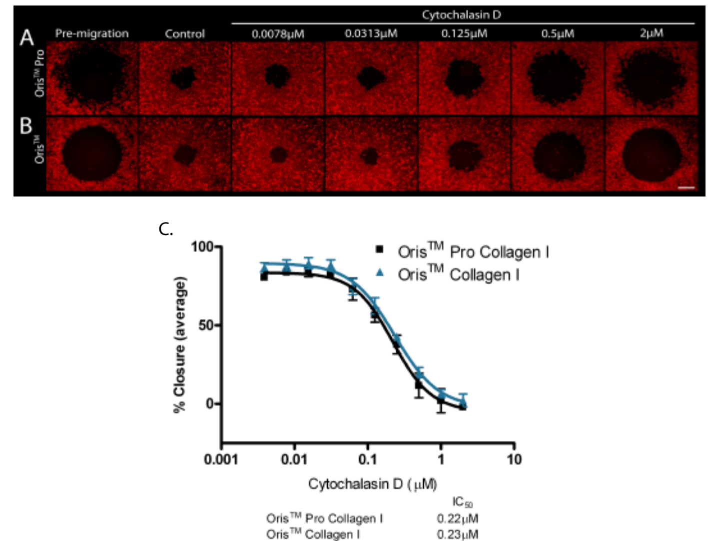


Figure 4. Determination of IC_{50} values for the actin polymerization inhibitor, Cytochalasin D, on the migration of HUVECs using the Oris™ Pro (A) and Oris™ (B) Assays. Cells were labeled with TRITC-phalloidin. Scale bar = 500 µm. Data are presented as the average percent closure of the Detection Zones ± SD from 4 wells for each condition. Results indicate overlapping dose-response curves and IC_{50} values between assay formats for Cytochalasin D treated cells seeded on Collagen I coated plates (C).

Conclusions

These experiments demonstrate substantial equivalence in performance of the Oris™ Pro and Oris™ Cell Migration assays using HT-1080 cells and HUVECs and three different classes of motility inhibitors. Overlapping trends were observed in cell migration and efficacy of inhibitors between the BCG and stopper-based assay formats. Furthermore, the presence of BCG did not interfere with cell viability and did not increase cytotoxicity. The self-dissolving BCG in the Oris™ Pro Cell Migration Assay makes the format conducive for the use of automated liquid handling equipment, streamlining assay set-up and reducing hands-on time. This assay is an attractive option for high content and high throughput screening for modulators of cell migration.

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