A Novel Cell Migration Assay for High Content Analysis

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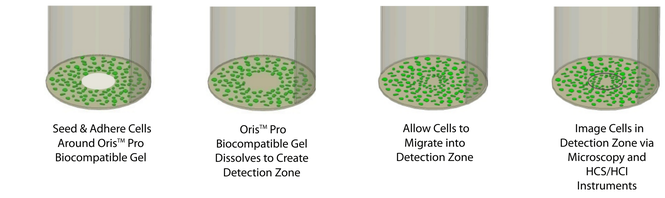
Abstract

Recent advances for screening modulators of cell migration include the development of assays based on establishing a monolayer of cells having a central cell-free zone into which cell migration can occur. This assay format allows an unobstructed view of cell motility throughout the duration of the experiment. We now describe a notable improvement to this assay format that utilizes a dissolving, non-toxic biocompatible gel (BCG) to form the cell-free zone on cell culture surfaces. Cells are seeded into 96-well plates that contain BCG centrally deposited in each well 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. Cell viability and cytotoxicity assays demonstrate that the dissolved BCG is non-toxic to commonly used cell lines including HT-1080, MDA-MB-231 and human umbilical vein endothelial cells (HUVECs). Dose responses using the actin polymerization inhibitor Cytochalasin D on HUVECs and HT-1080 cells were obtained using several High Content Screening (HCS) and High Content Imaging (HCI) instruments. Robust Z' factors of ≥0.5 are achievable with this assay, making it suitable for screening therapeutic compounds on tissue culture treated as well as Collagen I coated surfaces. This innovative assay format facilitates identification of therapeutic modulators of cell migration by enabling the use of automated liquid handling equipment and high content imaging platforms.

Oris™ Pro Cell Migration Assay Schematic



Materials and Methods

Cells and Reagents: HT-1080 fibrosarcoma, MDA-MB-231 breast epithelial, and PC-3 prostate carcinoma cell lines were obtained from the American Type Culture Collection (ATCC). Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza. Cytochalasin D was obtained from Enzo.

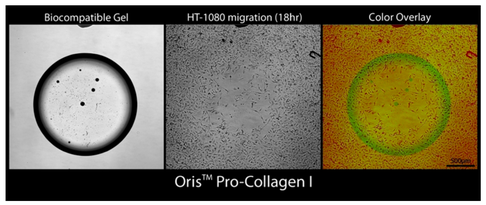
Cell Migration Assays: Oris™ Pro Assay wells were seeded with cell lines in 100µL of growth medium at densities between 25,000 and 30,000 cells/well as noted in the legends for specific experiments. The cells were allowed to attach for 2-4hr on Tissue Culture treated assay wells or 1hr on Collagen I coated assay wells prior to capturing pre-migration images. In certain experiments, Cytochalasin D was added to the media in 0.1% (v/v) DMSO as a vehicle.

After allowing cells to migrate for 18 to 24hr, all wells were fixed with 0.25% glutaraldehyde and labeled with DAPI (1:4000; 2-5 min) and TRITC-phalloidin (1:50; 45 min) + 0.1% TX-100 (in PBS). Phase or fluorescence images were acquired using a Zeiss AxioVert inverted fluorescence microscope and a BD Pathway™ 855 Bioimaging System. Using ImageJ analysis software (http://rsb.info.nih.gov/ij/index.html), the amount of migration was calculated from the acquired images based on area of closure. In other experiments, numbers of migrating cells were quantitated on DAPI stained cells using a TTP LabTech Acumen eX3 high content microplate cytometry system (TTP LabTech).

Cytotoxicity Assay: The Vybrant™ Cytotoxicity Assay (Molecular Probes) monitors the release of the cytosolic enzyme glucose 6-phosphate dehydrogenase (G6PD) from damaged cells into the medium and was performed as described by the manufacturer. Fluorescence was measured using a BioTek Synergy™ HT microplate reader using a 540/590 filter set.

Cell Viability Assay: The CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega) reagent, which measures metabolic function through reduction of formazan, was added directly to wells and was performed as described by the manufacturer. Absorbance was measured at 490nm using a BioTek Synergy™ HT plate reader.

BCG Dissolves to Reveal Detection Zone for Cell Migration

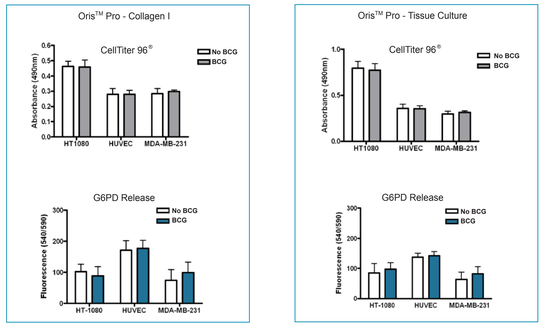


HT-1080 cells (30,000 cells in 100µL media) were seeded into an Oris™ Pro Collagen I coated well containing BCG. After 18hr migration, a phase contrast image was obtained and merged with an image of the initial BCG spot using a fiducial mark to align the images.

Results: HT-1080 cells migrate into the Detection Zone beyond the perimeter of the original BCG spot indicating that no residual BCG remains on the migration surface following its dissolution.

* Patent Pending

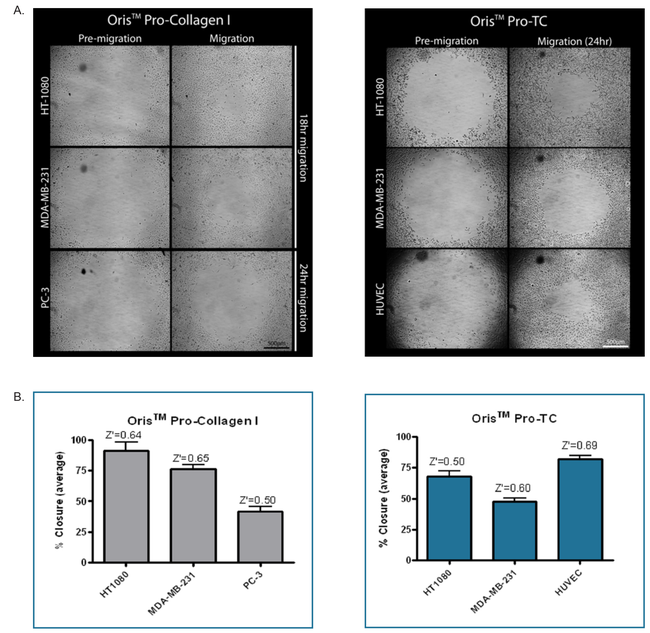
Non-Toxicity of Biocompatible Gel



Cell viability and cytotoxicity were measured using three cell types, HT-1080, MDA-MB-231, and HUVECs. Cell type specific media (50µL) was added to wells of the Collagen I or TC plates in the presence or absence of BCG and incubated for 4hr at 37°C/5% CO2. Cells (2,500 in 50µL media) were then added to all test wells and incubated for 18hr (Collagen I plates) or 24hr (TC plates) at 37°C/5% CO2. After 18-24hr, the Vybrant™ Cytotoxicity Assay (Molecular Probes) was used to measure the amount of glucose 6-phosphate dehydrogenase released from damaged cells. Cell viability was assessed using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega), which measures metabolic function through reduction of formazan. Fluorescence or absorbance were measured respectively for these assays using a BioTek Synergy™ HT microplate reader (n=14 +/- S.D.).

Results: BCG does not affect cell viability or cytotoxicity for 3 cell types tested on Oris™ Pro TC and Oris™ Pro Collagen I coated plates.

Robust & Reproducible Cell Migration



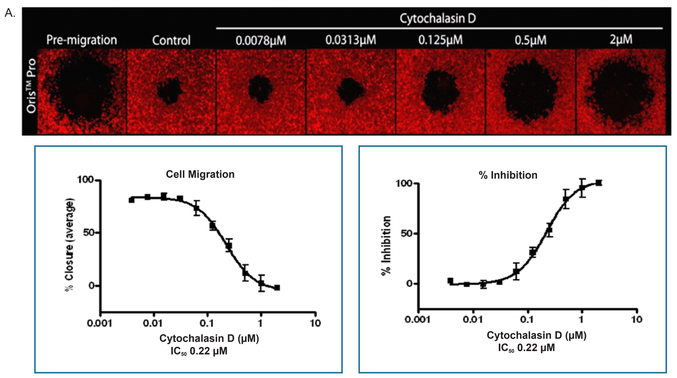
Cells (HUVEC: 25,000 cells/well; MDA-MB-231: 30,000 cells/well; HT-1080: 30,000 cells/well; PC-3: 25,000 cells/well) were seeded into Oris™ Pro Collagen I and TC assay plates and were allowed to attach for 1hr (Collagen I) or 4hr (TC).

(A) Phase images were acquired of the pre-migration time point (left column). Cell migration was permitted for 18-24hr (right column).

(B) Cell migration is presented as percent closure of the Detection Zone using ImageJ (n=24 +/- S.D.). For both Oris™ Pro TC and Oris™ Pro Collagen I, cells underwent robust cell migration. Z' factors, calculated as described by Zhang et al., J Biomol Screen. 1999;4(2):67-73., for all cell lines were ≥0.5, as indicated.

Results: Robust Z' factors using multiple compatible cell lines are achievable with Oris™ Pro Cell Migration Assays on Collagen I coated and TC surfaces.

Analysis of Cytochalasin D Effects on Cell Migration Using HCI and HCS Instrumentation



(A) HUVECs (25,000 cells/well) were seeded onto an Oris™ Pro Collagen I coated plate. A dose-response titration was performed using the actin polymerization inhibitor, Cytochalasin D. Cells were treated for 18hr, followed by fixation and staining with TRITC-phalloidin. Images were acquired using the BD Pathway™ 855 Bioimaging System. Cell migration was analyzed as the percent closure of the Detection Zone using ImageJ.

(B) HT-1080 cells (30,000 cells/well) were seeded onto an Oris™ Pro Collagen I plate. A dose-response titration was performed using Cytochalasin D. Cells were treated for 18hr, followed by fixation and staining with DAPI. Images were acquired and cell migration was analyzed by counting cells in a defined region-of-interest using an Acumen eX3 high content microplate cytometry system.

Dose-response curves were generated and IC_{50} values were calculated for each cell line as indicated (n=4 +/- S.D.).

Results: Oris™ Pro Assays are compatible with High Content Screening (HCS) and High Content Imaging (HCI) instruments to generate IC_{50} data for drug discovery.

Conclusions

Oris™ Pro's dissolving Biocompatible Gel (BCG) promotes the formation of defined Detection Zones and does not interfere with cell migration.

The presence of the BCG does not interfere with cell viability or cytotoxicity.

The Oris™ Pro Assay yields low variability and robust Z' factors using a broad range of cell types on Collagen I coated and TC surfaces.

The Oris™ Pro Assay is conducive for high throughput screening in drug discovery studies using HCI and HCS instrumentation.

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