Optimization of Cell Seeding Volume and Density for the Oris™ Pro 384 Cell Migration Assays

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Technical Memo

Summary

The Oris™ Pro 384 Cell Migration Assays represent the first-in-class 384-well cell migration assays. They enable high throughput screening (HTS) applications, are completely accessible to automated liquid handling equipment, allow for direct visualization of cells in real-time, and are suitable for high content analysis (HCA) with multiplexed phenotypic staining. The Oris™ Pro 384 Cell Migration Assays utilize a biocompatible gel (BCG) to establish a temporary and centrally-located cell-free zone in assay wells. With addition of culture media, the BCG dissolves to reveal a Detection Zone into which cells may migrate. The assay is simple to perform and yields highly reproducible results. We demonstrate herein that two critical parameters, the cell seeding volume and density, should be optimized for each cell type in order to establish clean Detection Zones at the onset of the Oris™ Pro 384 Cell Migration Assay.

Introduction

Cell migration plays an important role in physiological processes such as wound healing while its deregulation contributes to the pathology of cancer cell invasion and metastasis[1]. Agents that affect cell motility, either positively or negatively, have the potential to serve as therapeutics for wound healing or as anti-metastatic agents, respectively. Investigators engaged in high throughput screening of compounds seek improved, high-density format assays that offer 1) complete adaptability for liquid handling automation to facilitate ease of use and decrease hands-on time, 2) the ability to view cells in real-time during experiments, a feature that is lacking in Boyden chamber based assays where the membrane insert is an obstruction, and 3) greater reproducibility of data than in scratch assays where wound sizes can be inconsistent and cells and underlying extracellular matrix coating may also be damaged. Platypus Technologies has developed the Oris™ Pro 384 Cell Migration Assay, available with either tissue culture or collagen I coated surfaces, which are fully automatable, suitable for real-time viewing, and offer increased robustness over Boyden chamber and wound healing/scratch assays and compatibility with high content imaging (HCI) systems.

The Oris™ Pro 384 Cell Migration Assay (Figure 1) is a 384-well high density cell exclusion assay that will be useful for screening compound libraries. It is provided with deposits of biocompatible gel (BCG) in a portion of each well to exclude cells from adhering in the centers of the wells. The user simply seeds cells into the BCG-containing wells. The BCG is formulated to dissolve as the cells fall to the well bottom and begin to attach. Full dissolution of the BCG reveals a cell-free Detection Zone reproducibly positioned in the center of each well into which cells may then migrate. The cells may not be completely attached before the BCG dissolves, so the plates should be handled gently for an initial period of time (usually 1-2 hours on collagen I coated plates and 1-4 hours on tissue culture treated plates) to prevent dislodging cells into the Detection Zone. The experiments presented in this technical memorandum address how to optimize cell seeding volume and density to establish clean Detection Zones and permit robust migration to achieve high assay performance.

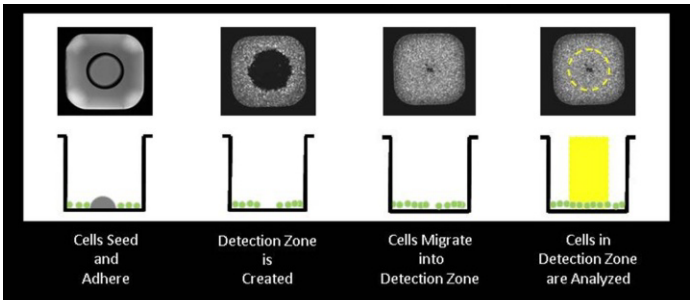


Figure 1. Schematic of Oris™ Pro 384 Cell Migration Assay

Materials & Methods

Cell seeding volume: HT-1080 fibrosarcoma cells were seeded at a density of 9,000 cells/well onto a Tissue Culture treated Oris™ Pro 384 Cell Migration Assay plate in varying volumes (20 µL – 100 µL) of media. Following a 90-minute incubation at 37°C/5% CO_2 to allow for attachment, cells were fixed with 0.25% glutaraldehyde and labeled with TRITC-phalloidin. Fluorescent images were captured, using a 2.5X objective on a Zeiss Axiovert inverted microscope equipped with a CCD camera, to observe the Detection Zones.

Cell seeding density: HT-1080 cells (5,000/well or 9,000/well) were seeded in 20 µL of media onto a Tissue Culture treated Oris™ Pro 384 Cell Migration Assay plate. Following a 2 hour incubation at 37°C/5% CO_2 to allow for attachment, cells were treated with 4 µM Cytochalasin D (pre-migration references) or 0.1% DMSO vehicle only

(migration wells). Following a 20 hour incubation to permit migration, cells were fixed and labeled with TRITC-phalloidin. Fluorescent images were captured using a 2.5X objective on a Zeiss Axiovert inverted microscope equipped with a CCD camera and the open area of the Detection Zones was calculated using ImageJ software from NIH.

Results

A principal feature of the Oris™ Pro 384 Cell Migration Assay is the BCG that initially excludes cells from attaching to the centrally located Detection Zone in the assay wells. The BCG rapidly dissolves into the cell culture media as the cells settle to the well bottom, attach and begin to spread out. It is important that cells are seeded in the proper volume and at the proper density to ensure that the Detection Zone is initially free of cells, i.e., clean. We conducted experiments to demonstrate the effects of different cell seeding volumes on the integrity of the Detection Zone. Results demonstrate that as the cell seeding volume is increased above 20 µL, the Detection Zone becomes less clean (Figure 2). These results indicate that cells seeded in larger volumes fail to settle to the bottom of the assay wells prior to the dissolution of the BCG and subsequently settle in the Detection Zone.

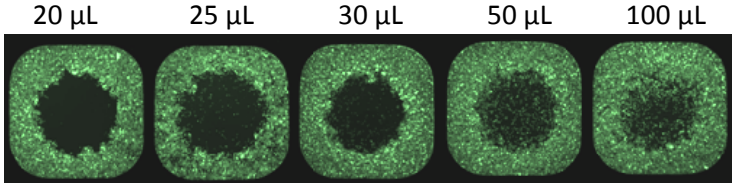
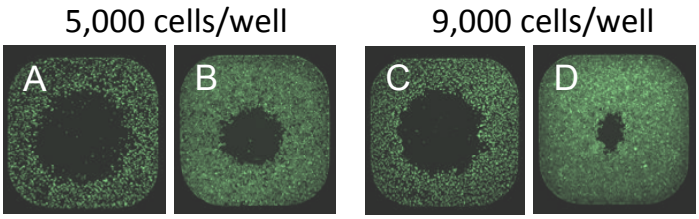


Figure 2. Effect of cell seeding volume on integrity of the Detection Zone in the Oris™ Pro 384 Cell Migration Assay. HT-1080 cells (9,000/well) were seeded in 20 – 100 µL of media, as noted. Following a 90 minute incubation at 37°C/5% CO_2 to allow for attachment, cells were fixed and labeled with TRITC-phalloidin (pseudocolored green). Fluorescent images were captured using a 2.5X objective on a Zeiss Axiovert inverted microscope equipped with a CCD camera to observe the Detection Zones. Results indicate that increasing the cell seeding volume above 20 µL leads to an unclean Detection Zone at the start of the assay.

We next examined the percent closure of the Detection Zone in the Oris™ Pro 384 Cell Migration Assay for cells that were seeded at 2 different densities. The results demonstrate that increasing the HT-1080 cell seeding density leads to increased migration of these cells and increased assay robustness as measured by the percent closure of the area of Detection Zone and Z' factor calculations[2], respectively. Figure 3 demonstrates that migration increases from 55.9% closure for 5,000 cells/well to 89.3% closure for 9,000 cells/well. A careful balance of seeding density should be sought. For example, cells that are seeded at too low of a density to form a confluent monolayer would fail to migrate in a timely fashion (data not shown). Conversely, cells seeded too densely in an assay well would fail to securely adhere to the plate and would settle in the Detection Zone at the beginning of the assay.



55.9% ± 4% closure
Z' = 0.50

89.3% ± 2% closure
Z' = 0.88

Figure 3. Effect of cell seeding density on the migration of HT-1080 cells in the Oris™ Pro 384 Cell Migration Assay. HT-1080 cells at 5,000/well (A and B) or 9,000/well (C and D) were seeded in 20 µL of media. Following a 2 hour incubation at 37°C/5% CO_2 to allow for attachment, cells were treated with either 4 µM Cytochalasin D as pre-migration references (A and C) or 0.1% DMSO vehicle (B and D) as migration wells, respectively. Following a 20 hour incubation to permit migration, cells were fixed and labeled with TRITC-phalloidin (pseudocolored green). Fluorescent images were captured using a 2.5X objective on a Zeiss Axiovert inverted microscope equipped with a CCD camera and the open area of the Detection Zones was calculated using ImageJ. Data are presented as the average percent closure of the Detection Zones ± SD from 4 wells/condition. The results demonstrate that increasing the HT-1080 cell seeding density leads to increased migration of these cells as measured by the percent closure of the area of Detection Zone and increased robustness of the assay as measured by Z' factor calculations.

Conclusions

While the assay is simple to perform, care must be taken to minimize the cell seeding volume and optimize the cell seeding density to achieve robust results when performing the Oris™ Pro 384 Cell Migration Assay. The use of a dissolving BCG in the Oris™ Pro 384 Cell Migration Assay makes the format conducive for automated liquid handling equipment thereby streamlining assay set-up and minimizing hands-on time. This first-in-class 384 well high density cell migration assay is an attractive option for high throughput screening and high content analysis for modulators of cell migration.

References

[1]Vogt, A. (2010) Advances in two-dimensional cell migration assay technologies. European Pharmaceutical Review, 5:26-29.

[2]Zhang, JH, Chung, TDY, Oldenburg, KR. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. Journal of Biomolecular Screening, 4:67-73.