Establishing Pre-Migration References in the Oris™ Pro Cell Migration Assay

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Application Note

ORIS™ PRO CELL MIGRATION ASSAY

Introduction

Cell migration is critical for many physiological and pathological events, such as embryonic development, wound healing, the inflammatory response, and tumor metastasis (1). The Oris™ Pro Cell Migration Assay (Figure 1) employs a 96-well plate in which a non-toxic, dissolving biocompatible gel (BCG) is deposited in each well to exclude cells from attaching to the centers of the well. After cells have adhered and the BCG has dissolved to reveal a cell-free zone, cells are able to migrate into the central part of the well (i.e. Detection Zone). Since cell movement into the Detection Zone can occur once the BCG dissolves, it can be challenging to obtain a pre-migration reference control (e.g. starting point) necessary for quantifying cell migration.

This application note describes a method to quantify cell migration using two different approaches to obtain pre-migration references. The first approach utilizes fixed pre-migration reference wells while the second approach uses a pharmacological inhibitor of cell migration to create reference controls.

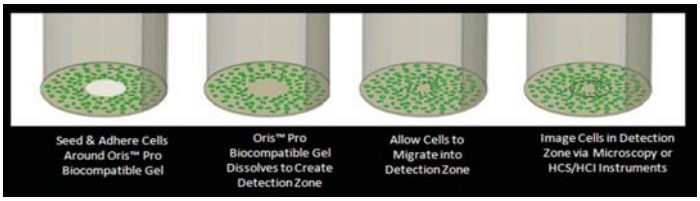
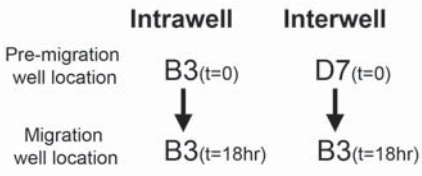


Figure 1. Oris™ Pro Cell Migration Assay Schematic

Approach 1: The use of Fixed, Pre-migration References to Quantify Cell Migration

Methods: In this approach, cell migration was calculated using separate, fixed pre-migration reference wells. To determine the validity of this approach, the cell migration data obtained using interwell calculations (i.e., cells fixed at pre-migration compared to separate cells fixed following migration) was compared to cell migration data obtained using intrawell calculations (i.e., cells in the same well imaged live at time 0 and fixed following migration). HT-1080 fibrosarcoma cells were seeded on an Oris™ Pro Collagen I plate and permitted to attach for 1 hour at 37°C/5%CO_2. A subset of wells were designated as interwell pre-migration references and fixed with 0.25% glutaraldehyde. Using a 5X objective on a Zeiss Axiovert 200 inverted microscope equipped with a CCD camera, phase contrast images were captured of these interwell pre-migration reference wells, in addition to those wells designated as intrawell pre-migration reference wells. After image capture, the plate was returned to 37°C/5%CO_2 for cell migration to proceed. Migration was terminated after 18 hours by fixing cells in remaining wells with 0.25% glutaraldehyde, and images were captured. The migration image set was used to compare both the interwell and intrawell cell migration data sets (see following diagram).

Note: Depending upon fixation method, care should be taken to ensure fixative vapors do not compromise the viability of cells in other wells of plate. As an alternative, a separate reference plate can be used.



Using Muscale CMA_{cfz} image analysis software (Phoenix, AZ), the area of the Detection Zone was measured for both of the pre-migration conditions (fixed and live) and the migration timepoint. Cell migration was determined as percent closure, calculated as follows:

$$\left(\frac{(\text{Pre-migration})_{area} - (\text{Migration})_{area}}{(\text{Pre-migration})_{area}}\right) \times 100$$

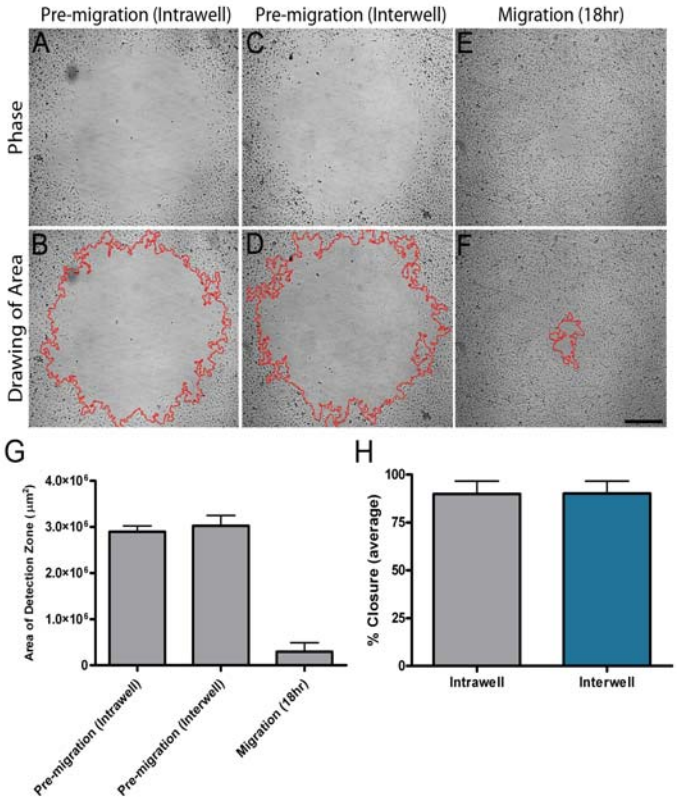


Figure 2. Comparison of HT-1080 cell migration quantified from intrawell and fixed, interwell pre-migration reference wells. A-F, Representative phase images and corresponding area drawings of the Detection Zone using Muscale CMA_{cfz} image analysis software of intrawell pre-migration control (A and B), interwell pre-migration control (C and D), and 18 hours after cell seeding (E and F). Scale bar = 500µm. G, Areas of the Detection Zones for the pre-migration and migration time points. H, Cell migration expressed as % closure calculated from either the intrawell pre-migration dataset or the interwell pre-migration reference wells. The equation to calculate % closure is described in the methods section. Data are presented as % closure ± SD from 24 wells for each condition.

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Results: The Oris™ Pro Cell Migration Assay contains BCG that is uniformly deposited in well centers to produce consistently-sized Detection Zones in all wells within and between plates. As a result, quantification of cell migration using images of same-well, live pre-migration references captured one hour post-seeding (intrawell) was compared to that using discrete, fixed pre-migration references (interwell) in conjunction with fixed migration wells. Phase images and area drawings of HT-1080 cells demonstrate similarities in the size of the Detection Zones for both the intrawell and interwell pre-migration conditions (Fig 2A-D and G). When percent closure was calculated using the 18 hour migration image set (Fig 2E and F), the data were substantially similar between the two conditions (Fig 2H). Using either the intrawell or interwell pre-migration reference options, HT-1080 cells migrated to 91% closure (intrawell: 91.0% ± 7.0 vs. interwell: 91.2% ± 6.6). These data demonstrate the validity of using the fixed, interwell pre-migration reference option to quantify cell migration in the Oris™ Pro Cell Migration Assay.

Approach 2: The use of Cytochalasin D-treated Pre-migration References to Quantify Cell Migration

Methods: In this approach, cell migration was calculated using Cytochalasin D (CD)-treated wells. To determine the validity of this approach, the cell migration data obtained using the CD-treated wells was compared to cell migration data obtained using fixed (interwell) pre-migration wells. Human umbilical vein endothelial cells (HUVECs) were seeded on an Oris™ Pro tissue culture treated plate and were permitted to attach for 2 hours at 37°C/5%CO_2. A subset of wells was then designated as interwell pre-migration references and fixed with 0.25% glutaraldehyde. An additional subset of wells was designated as cytochalasin D references and 1µM CD (Enzo Life Sciences) or vehicle (DMSO) control was added to these wells. The plate was returned to 37°C/5%CO_2 for migration to proceed. Migration was terminated after 24 hours by fixing cells in remaining wells with 0.25% glutaraldehyde, followed by staining with 0.2µM TRITC-phalloidin (Sigma) in 0.1% Triton X-100.

Fluorescence images were captured using a 2.5X objective on a Zeiss Axiovert 200 inverted microscope equipped with a CCD camera. Using the Muscale CMA_{cfz} software, cell migration was calculated and the results were compared for the CD-treated references and the fixed, interwell pre-migration references.

Results: A drug known to block cell migration can be employed to create the pre-migration reference. Figure 3 compares 24-hour cell migration quantified from fixed, interwell pre-migration references to that calculated using cells treated with an actin polymerization inhibitor, cytochalasin D. The Detection Zones of wells treated with 1µM CD and the fixed, interwell pre-migration reference wells were indistinguishable (Fig 3A-D and G). When migration was calculated using the 24-hour migration image set (Fig 3E-F), the data between the two pre-migration options were substantially similar (interwell: 76.3% ± 3.1 vs. CD-treated: 76.7% ± 3.3). These (Fig 3H). These results demonstrate the applicability of using a drug treatment as an alternative to cell fixation to establish a reference value for quantifying cell migration in the Oris™ Pro Assay.

Conclusions

This application note provides two approaches to obtain pre-migration references suitable for quantifying cell migration in the Oris™ Pro Cell Migration Assay. Using Muscale CMA_{cfz} image analysis software to quantify the area of the Detection Zones under different conditions, it has been demonstrated that a fixed, interwell pre-migration reference control produces comparable data as an intrawell pre-migration reference control. Additionally, a drug that inhibits cell migration, such as cytochalasin D, can also serve a reference option to calculate percent closure. These results illustrate that multiple approaches can be employed to establish pre-migration references in the Oris™ Pro Cell Migration Assay.

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

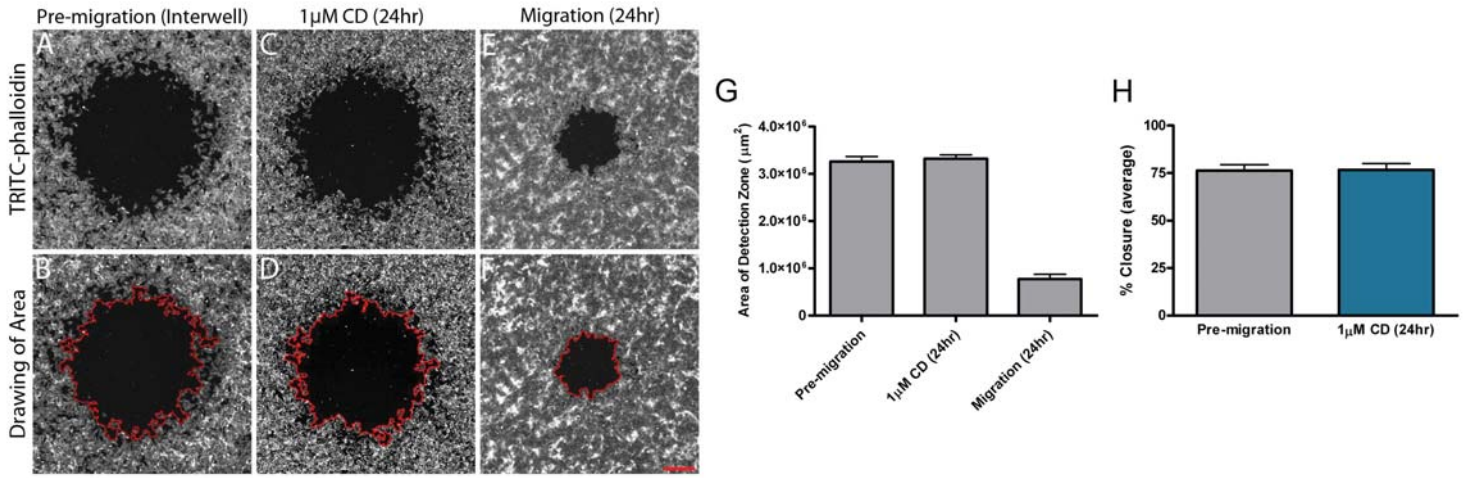

Figure 3. Quantification of cell migration using cytochalasin D-treated pre-migration references. Representative images and corresponding area drawings of either interwell pre-migration (A and B) or cytochalasin D (CD) pre-migration wells (C and D). Representative images of HUVECs that have migrated for 24 hours in the presence of vehicle control (E and F) on an Oris™ Pro tissue culture treated plate. Cells were fixed and stained using TRITC-phalloidin. Scale bar = 1000µm. G, Area of Detection Zone for the pre-migration and migration conditions. H, Comparison of cell migration quantified using either the interwell pre-migration references or 1µM CD-treated wells. Data are presented as % closure ± SD from 16 wells for each condition.

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