Investigation of a Label Free Higher Density Cell Migration Assay using an Automated Microplate Imager and Automated Data Analysis Software

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Abstract

Complex biological or environmental signaling events can lead to cell migration, the movement of cells from one location to another. The migration of cells commonly occurs during both normal biological processes such as the development of an organism or marking pathological events such as cancer metastasis. This study used a high density cell exclusion assay in 384-well format for investigation of cell migration using a GFP-expressing breast cancer cell line. This study compared the use of a high density cell exclusion assay in 384-well format for investigation of cell migration using both a conventional tracking dye and label-free detection. The use of a biocompatible gel (BCG) deposited in a portion of the well was used to exclude cells during seeding. A cell free zone was revealed after dissolution of the BCG following media/cell addition to the wells. After cell attachment, compound addition can be made directly to the wells for investigation. An automated cell imaging microplate reader was used to determine the % confluence of cells migrating into the cell free zone using an algorithm integrated in the instrument control software.

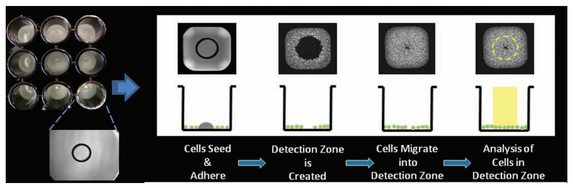

Figure 1. Oris™ Pro Cell Migration Assays. The Oris™ Pro Assay uses a non-toxic biocompatible gel, BCG to form a cell-free detection zone on culture surfaces. After cells are seeded into the 96-or 384- well migration assay plates, the BCG dissolves permitting cells to migrate into the well centers. Images of cells in the central detection zone can be captured and quantified in real-time using microscopes or imaging microplate readers.

BioTek® Instrumentation

Figure 2. Cytation™5 is a cell imaging multi-mode microplate reader that combines automated digital microscopy and conventional multi-mode microplate detection.

Materials and Methods

Cell Culture

MDA-MB-231/GFP cells were grown to ~80-90% confluence in T-75 tissue culture flasks in Advanced DMEM (Gibco Cat. No. 12491-015) supplemented with 10% FBS, 2 mM L-glutamine, 1% Pen-Strep.

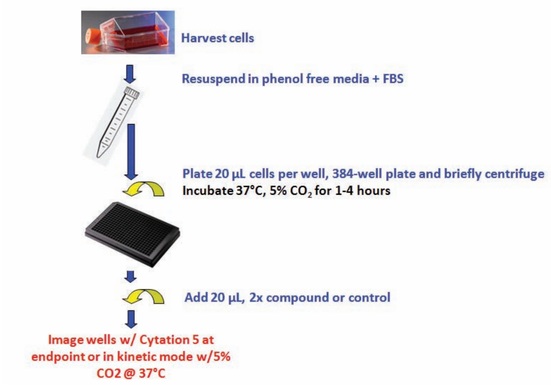

Figure 3. Assay workflow. MDA-MB-231/GFP cells were harvested and prepared for seeding. MDA-MB-231/GFP expressing cells were plated in phenol-free media.

Materials and Methods (cont.)

Optimization of Cell Seeding Density

MDA-MB-231/GFP cells were diluted to the appropriate cell plating density in PFCM.

For the **Oris™ Pro 384 Cell Migration Assay**- Collagen I Coated, 20 µL of MDAMB-231/GFP were seeded in replicates of six (6) at densities of 5, 7.5, 10, 12.5, 15 and 17.5K cells/well.

The assay plate was centrifuged briefly at 1000 rpm in an Eppendorf 5810R centrifuge immediately following the addition of cells to facilitate seeding.

Cells were allowed to attach for a minimum of 1 hour in a humidified incubator at 37 °C, 5% CO_2 prior to compound addition[2,3].

Half of the wells at each cell density were treated with cytochalasin D (CD) to a final concentration of 1 µM while the other half were treated with vehicle alone (0.1% DMSO/PFCM).

Cells were imaged kinetically for up to 36 hours in a **Cytation™5 microplate imager** with incubation at 37 °C and a gas control module set to 5% CO_2 using the settings outlined in Table 1 and described in further detail below.

Images were analyzed using **Gen5™ Data Analysis** (BioTek Instruments, Inc., Winooski, VT), Microsoft Excel (Redmond, CA), or GraphPad Prism® (La Jolla, CA).

Gen5™ Data Analysis software was used to apply a disc-shaped mask revealing the detection zone. The percent brightfield was determined in detection zone for final analysis.

A comparison of percent confluence of the detection zone of post-migration wells with control wells in which no migration occurred was used to calculate post-migration percent closure using imaging data[4].

Cytation 5 Read Parameters	
Plate type	384-well
Mode	Image
Objective	2.5X
Color	GFP
Exposure	Auto
Horizontal offset	0 µm
Verticle offset	-406 µm

Table 1. Cytation™5 Read Parameters. The 384-well plate format required a vertical offset to insure the cell free zone was centered in the image field.

Cytochalasin D Dose Response

Cells were harvested and seeded at optimal density as described above in 384-well Oris™ Pro microplates.

Following the 1 hour incubation period cytochalasin D was serial diluted 1:3 and transferred to the assay plate in triplicate. Plates were imaged and analyzed as described above.

Results and Discussion

Optimization of Cell Seeding Density: Oris™ Pro 384-well Assay

The optimum cell seeding density was determined by treatment with either the known migration inhibitor, 1µM cytochalasin D, or vehicle alone.

Kinetic data allowed the optimal incubation time and seeding density to be determined for further experiments (Figures 4&5).

Percent confluence calculated using Gen5™ was used to determine the seeding density giving the most reproducible results for use in future experiments.

MDA-MB-231/GFP cells plated at a density of 12,500 cells/well and incubated at 36 hours gave optimal results.

Cells were then used at the optimal seeding density for dose response experiments using the migration inhibitor cytochalasin D.

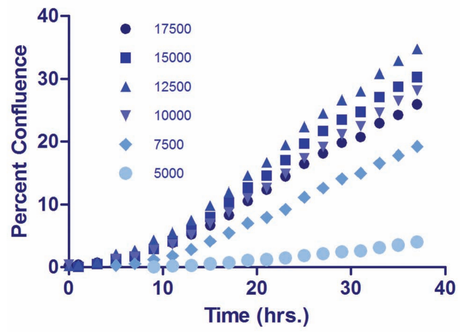

Figure 4. Seeding density and incubation time optimization. Cells were plated at the indicated seeding density in a 384-well plate format and imaged every two hours.

Results and Discussion (cont.)

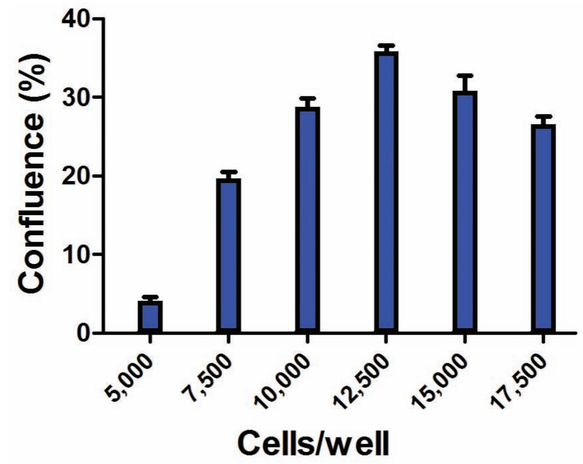

Figure 5. Seeding density and incubation time optimization. Percent confluence was calculated at each cell density to determine optimal cell seeding density and adequate incubation time (representative data shown at 36 hrs.post-seeding).

Cytochalasin D Dose Response

Cells were treated with CD in a dose response format in triplicate and allowed to migrate for the appropriate length of time.

Data analysis was performed using the methods described above for determination of IC_{50} values (Figure 6).

The IC_{50} value for treated cells was 57.0 nM when determined by Gen5™ using percent confluence.

Analysis by calculation of percent closure of the detection zone showed equivalent results.

These determinants showed acceptable correlation between the two analytical methods employed as well as with previously published data[3].

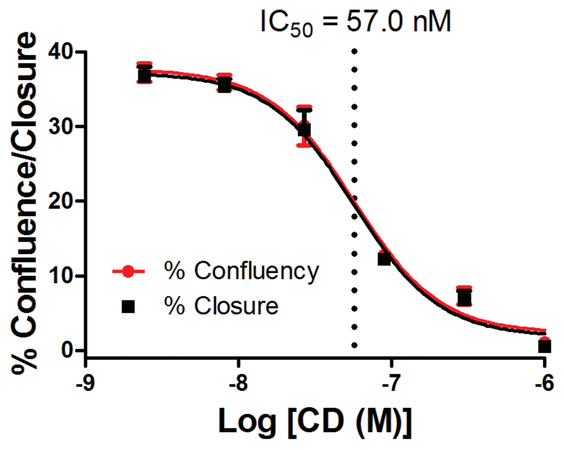

Figure 6. Dose response curves. MDA-MB-231/GFP cells were imaged in the 384-well microplate format after treatment with CD in dose response format following a 36 hour incubation. Plot depicts percent confluence or percent closure vs. inhibitor concentration. The resultant IC_{50} determinants were identical.

Conclusions

1. The **Cytation™3 Cell Imaging Multi-Mode Reader** provides a suitable platform for quantifying cell migration assays using systems such as **Oris™ Pro migration assays**.
2. The Oris™ Pro cell migration assay is a robust, easily automatable cell based assay that provides complete visual access to the cells and is suitable for high throughput screening.
3. The quantification of extent of cell migration can be performed using automated data analysis tools embedded in the Gen5™ Image+ operating software giving comparable results to determinations using the more commonly used readout of percent closure.

[1]Comley, J. Cell Migration: probing cell movement with smarter tools. (2012/13). Drug Discovery World. Winter; Vol 14, Issue 1, pp. 33-51. [2]Oris™ Pro Cell Migration Assay -Collagen I Coated 'Protocol & Instructions' (2013), Platypus Technologies, LLC., Madison, WI. Fronczak, J.A., Hulkower, K.I. and Vogt, A. (2011, May) A robust 384-well cell migration assay for high content analysis of cells treated with anti-cancer therapeutics. Poster session presented at American Association of Pharmaceutical Scientists, Washington, DC. [4]Hulkower, K.I. and Gehler S.R. How to measure area closure using the Oris™ Pro cell migration assay Technical Memo. Platypus Technologies, LLC. Madison, WI.