Cell Migration Assays from Platypus Technologies (Oris™ and Oris™ Pro) are Digitally Imaged Using the PHERAstar *FS* from BMG LABTECH

Carl Peters[1] and Gopal Krishnan[2] [1]BMG LABTECH, Cary, NC [2]Platypus Technologies, Madison, WI

Abstract

The Oris™ Cell Migration Assay in a 96-well format represents a clear improvement in cell migration analysis over the traditional 'scratch' test which can be variable from lab to lab and can disrupt extracellular matrix which is vital for cell migration. The Oris™ Pro Cell Migration Assay uses a non-toxic, dissolvable biocompatible gel (BCG) to form cell exclusion zones on cell culture surfaces. After seeding cells into the 96-well plate, the BCG dissolves, permitting cells to migrate into the well centers. This represents a further advancement that allows for high-throughput cell migration analysis to be performed in 384-well plates. The PHERAstar *FS* proves to be an excellent HTS microplate reader to assess these cell migration assays. The direct optic bottom reading system on the PHERAstar *FS* permits high resolution, cell layer well scanning. This feature provides a digital image and the opportunity to visualize as well as quantify the cell migration exhibited by the Oris™ and Oris™ Pro products.

Introduction

Cell migration is a vital part of a number of normal physiological processes such as:

- Wound healing
- Immune-cell trafficking
- Embryonic and nervous system development

Cell movement that is not properly regulated is associated with diseases, most notably cancer where one of the indicators of poor prognosis is tumor metastasis.
In order to study cell migration and its role in these phenomenon it is necessary to have assays which accurately quantify cell migration events. The 'scratch test' has traditionally been employed to visualize cell migration by creating a cell free gap in a layer of confluent cells. The common signaling pathways associated with cell migration have been identified using this approach, although it is less than perfect. The main liability to the 'scratch test' is that the process of scratching also disrupts the extracellular matrix, which is essential for cell migration. Furthermore, lab to lab variation in the technique employed to create the scratch leads to variability in results and consequently confusion within the literature.

Materials and Methods

Assay Principle

The Oris™ and Oris™ Pro Cell Migration Assays offer an alternative to the 'scratch test'. They employ a stopper or a biocompatible gel to create the cell free gap in the center of a well in a microplate. Cells which are seeded into each well are initially restricted from adhering to the center of each well creating a 'detection zone' (Figure 1).

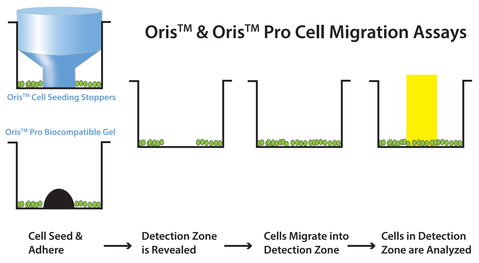

Figure 1 Oris™ and Oris™ Pro cell migration improve on 'scratch test' and enhance throughput

Following cell seeding the cell free detection zone is revealed. The cells can then be treated with a compound of choice and cell migration is assessed by the amount of cells which enter this detection zone. When cells are either labelled with a fluorescent probe or fixed and stained they can be visualized with a microplate reader such as the PHERAstar *FS* from BMG LABTECH.

Materials

- Oris™/Oris™ Pro Cell Migration Assays (96 & 384 well, black , clear bottom plates
- PHERAstar *FS* from BMG LABTECH (Figure 2)

Experimental Design

Cells were seeded into Oris™ 96 well plates or Oris™ Pro 96 or 384 well plates. Oris™ employs a stopper which was removed after cells adhered to the plate while a biocompatible gel used in Oris™ Pro. After seeding, cells were treated with varying concentrations of cytochalasin D. Alternatively, DMSO was applied as a vehicle control. Cells were fixed and stained at the conclusion of the experiment with FITC.

Cell migration was visualized and quantified by detection of FITC staining using the PHERAstar *FS* which was setup to read the plates using the bottom optics and a single emission fluorescence intensity module that was fitted with a ex485 excitation filter and an em520 emission filter. A well scan reading mode was employed that read a 10 x 10 matrix in each well. The scan width was 5 mm when 96 well plates were used and 3 mm when 384 well plates were used.

After well scan data was obtained, fluorescence intensity in the detection zone was quantified within MARS, the data analysis software supplied by BMG LABTECH. Using MARS we were able to define the scan diameter used as 2 mm (Figure 3), equal to that defined by either the cell seeding stoppers or the biocompatible gel used in the Oris™ or Oris™ Pro Cell Migration Assays, respectively.

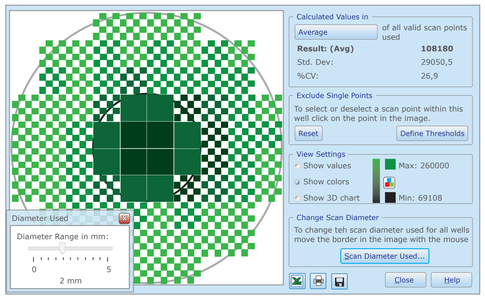

Figure 3 Analysis of Well Scan Data in MARS
Using MARS data analysis software allows users to evaluate Well Scan Data in a variety of ways. Data can be visually evaluated with different color schemes or 3-D representations. Data used can be defined by adjusting the diameter range as shown here. Numerical outputs can be analyzed using a wide variety of statistics and relationships portrayed with various curvilinear models.

Results and Discussion

A clear trend that meets expectations is revealed in the digital images obtained from well scan data. This can be seen with either Oris™ or Oris™ Pro 96 well plates (Figure 4 and 5).

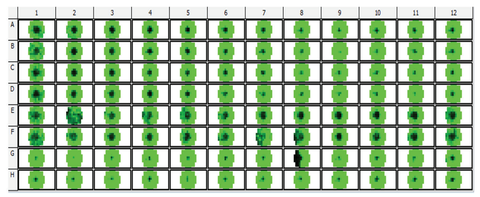

Figure 4 Well Scan Data obtained of the PHERAstar *FS* using Oris™ 96 well plates
In rows A to D columns 1 to 12 cells have been treated with decreasing amounts of cytochalasin D. Column 1 = 2 µM cytochalasin D, column 11 = 2 nM cytochalasin D, column 12 = 0.1% DMSO (ie vehicle control)
Rows E and F are negative control (2 µM cytochalasin D)
Rows G and H are positive control (0.1% DMSO)

The Oris™ 96 well plates in this experiment exhibit some minor abberations with regard to cell density which likely represent user error in the fixing and staining steps. Despite these discrepancies encroachment into the detection zone is clear, as cytochalasin D decreases cell migration into the detection zone increases.

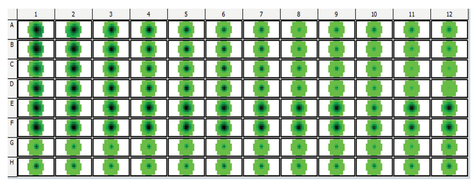

Figure 5 Well Scan Data obtained of the PHERAstar *FS* using Oris™ Pro 96 well plates
In rows A to D columns 1 to 12 cells have been treated with decreasing amounts of cytochalasin D. Column 1 = 2 µM cytochalasin D, column 11 = 2 nM cytochalasin D, column 12 = 0.1% DMSO (ie vehicle control)
Rows E and F are negative control (2 µM cytochalasin D)
Rows G and H are positive control (0.1% DMSO)

The Oris™ Pro 96 well plates in this experiment exhibit a very consistent appearance and encroachment into the detection zone is very clear, as cytochalasin D decreases cell migration into the detection zone increases.

The amount of cell migration into the detection zone could be quantified by taking the average fluorescence intensity observed in the 2 mm detection zone as defined in the MARS data analysis software. In figure 5 these average fluorescence intensity values were graphed with respect to their corresponding concentration of cytochalasin D.

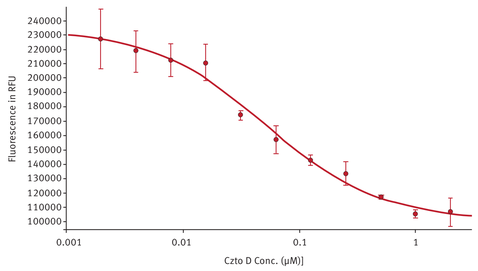

Figure 6 Curvilinear comparison of average fluorescence and cytochalasin D concentration in the Oris™ Pro 96

The increase in fluorescence signal intensity correlates with a decrease in cytochalasin D concentration. This relationship is well represented by a 4-parameter fit curve (r2 = 0.987)

The use of a biocompatable gel to define the 2 mm detection zone in the Oris™ Pro Cell Migration Assay enables the use of the more densely packed 384 well format. We found the performance of the 384 well format to rival that seen in the 96 well format (data not shown)

About PHERAstar

The PHERAstar *FS* has a number of attributes that aid performance of cell-based assays such as the Oris™ and Oris™ Pro.

- True, direct free-air optical path for bottom reading
  - ⇨ No fiber optic bundles or light guides
- Software controlled mode switching
  - ⇨ User intervention not necessary
- Automatic Z-Height focusing at 0.1 mm resolution
  - ⇨ Precisely focuses light onto the cell monolayer
- Same assay modules used for top or bottom detection
  - ⇨ No additional dichroics, mirrors or filters needed
- Well scanning for high resolution cell layer detection
  - ⇨ Digital imaging for visualization and quantification

Figure 2 The PHERAstar FS from BMG LABTECH

Conclusion

- High resolution well-scanning using the PHERAstar *FS* provides digital imaging which can be used to visualize and quantify Oris™ and Oris™ Pro (96 & 384 well) Cell Migration Assays
- Oris™ and Oris™ Pro Cell Migration Assays provide consistent results without the need for mechanical scratching
- Higher through put is now attainable with the Oris™ Pro 384 well Cell Migration Assay
- MARS data analysis assists in both visual and quantification analysis

Find more information at
Booth #921