#4137 An improved assay system for cell migration and invasion

Jeff Partridge, Stephen Rimsa, Paula Flaherty; Corning Life Sciences, Bedford, MA

Abstract

In vitro cell migration and invasion assays are frequently used as model systems for quantifying the directed movement of cells towards a chemoattractant stimulus, or to measure how a particular drug, antibody or extra cellular matrix (ECM) coating affects that movement. Analysis of this movement is often accomplished through the use of visual methods that are either time-consuming, labor-intensive, or subjective. To address these issues, an improved fluorescence blocking version of the Boyden chamber was developed. As cells migrate or invade through a unique fluorescence blocking microporous membrane, they can be detected using a bottom-reading spectrofluorometer or fluorescence microscope. Cells remaining in the upper chamber of the insert are shielded from this detection, allowing for quantitation of cell numbers in a homogeneous assay system.

The technology blocks ≥ 98% transmission of light from 360 - 690 nm as measured by transmission spectrophotometry, and improvements have been made over previous embodiments such that >50% more light in the range of 360 - 490 nm is blocked. These enhancements now permit the use of the blue fluorescent Hoechst dyes and DAPI for direct cell enumeration and multiplexing. Functional applications of this technology were demonstrated in several well-established assays: endpoint staining in a model tumor invasion system yielded a Z'-factor >0.5; real-time chemotaxis of monocytes demonstrated a peak signal between 25-35 minutes; and migration of human endothelial cells to VEGF was demonstrated with high reproducibility (CV < 20%). Additionally, the measured IC50 values of chemotherapeutic compounds including paclitaxel and doxorubicin remained consistent with previously reported results.

Corning® FluoroBlok™ cell culture inserts can efficiently be used for high-throughput cell migration studies, are automation friendly and yield highly reproducible results.

Introduction

While the Boyden chamber is an example of a 'classic' assay system used to study the events surrounding cell migration and invasion *in vitro*, its usefulness continues to be demonstrated. In fact, the thousands of references that pertain to its use reinforce its ability to answer many diverse questions surrounding cell migration. Some novel examples include use of this assay to assess molecules involved in invasion, immune cell effects on invasion, and enrichment for cancer stem cells.[1-2]

Methods

Transmission Spectrophotometry

Total transmission values of unbonded Corning FluoroBlok membranes were measured using a HunterLab UltraScan® PRO. Membranes were immersed in commonly used biological solvents (water, saline, DMSO in culture medium and paraformaldehyde) for up to 24h and values were remeasured.

Tumor Cell Migration, Invasion and IC50 Assays[4-6]

HT-1080 (a human fibrosarcoma cell line) and 3T3 (mouse embryonic fibroblasts) cells were seeded at 25,000 cells/well. Chemoattractant was 5% FBS. For overnight kinetic assays, an Aldrich® AtmosBag was used to maintain a 5% CO_2 atmosphere. RFU values were obtained with a Perkin Elmer EnVision® or Tecan Safire²™. For IC50 assays, doxorubicin (data not shown), paclitaxel, and doxycycline were purchased from Sigma-Aldrich.

Leukocyte Chemotaxis[7]

THP-1 cells (a monocytic cell line) were seeded at 100,000 cells/well on fibronectin coated inserts and chemotaxis to MCP-1 (R&D Systems®) was measured.

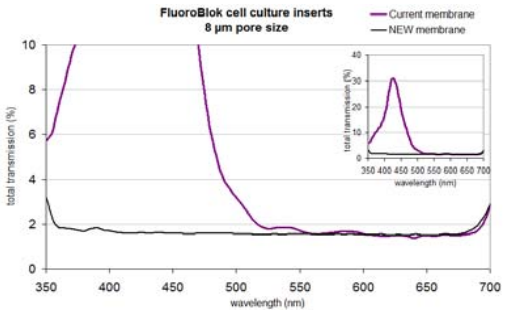
Endothelial Migration[9]

Starved HUVEC (primary endothelial cells, Lonza) were seeded at 100,000 cells/well on fibronectin coated inserts. Migration response to 10 ng/ml VEGF (Corning Life Sciences) was measured (data not shown).

Migration refers to directed cell movement in general. Invasion refers to degradation of a 3D physical barrier, such as an extracellular matrix or basement membrane, *i.e.*, Corning Matrigel®[2]

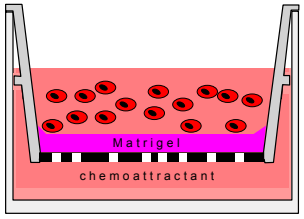
All procedures used 24 well individual or multiwell Corning FluoroBlok inserts. Tumor cell migration, invasion and IC50 assays utilized 8 µm pore size inserts. Leukocyte chemotaxis and endothelial migration utilized 3 µm pore size inserts. Cells were stained with calcein AM unless otherwise indicated.

Figure 1. Transmission Spectra of FluoroBlok Membranes



Total transmission of 8 µm pore size FluoroBlok membrane. Inset details expanded y-axis. Effects of commonly used solvents on total transmission were negligible.

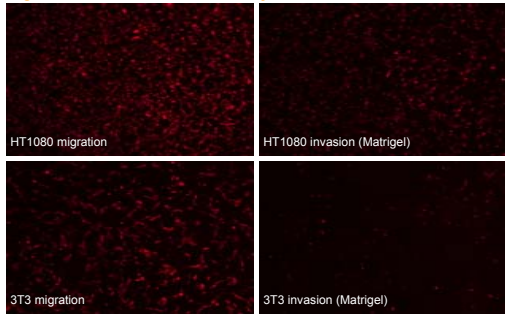
Figure 2. Fluorescence Blocking Boyden Chamber



New membrane

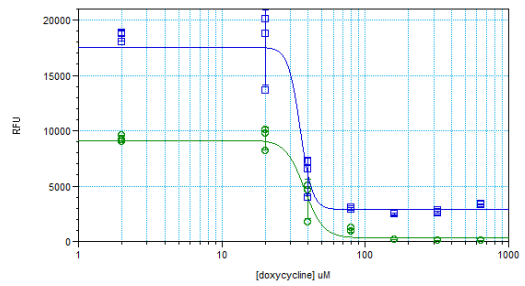
Schematic of Boyden chamber invasion assay with cells added to apical chamber.

Figure 3. Tumor Invasion Assay - Kinetic



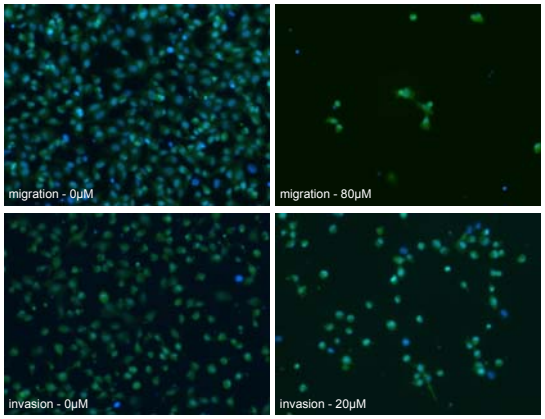
HT-1080 and 3T3 cells pre-labeled with $DiIC_{12}(3)$. HT-1080 cells are capable of migration and invasion. Lacking matrix metalloproteinases, 3T3 cells can migrate but not invade. The Z'-factor for this assay system > 0.5, which identifies an excellent screening assay;[3] the invasion index is typically > 5.

Figure 4. Migration Assay - Doxycycline IC50s by Label



HT-1080 cells labeled post-migration with Hoechst 33342 (blue) and calcein AM (green). IC50s calculated by label: H33342, 35 µM; calcein, 38 µM; previously measured at 45 – 61 µM.[5-6]

Figure 5. Multiplexing of Fluorescent Labels



HT-1080 cells were post-labeled with Hoechst 33342 (blue) and calcein AM (green) after migration or invasion. Cells were treated with doxycycline in the apical and basolateral chambers.

References

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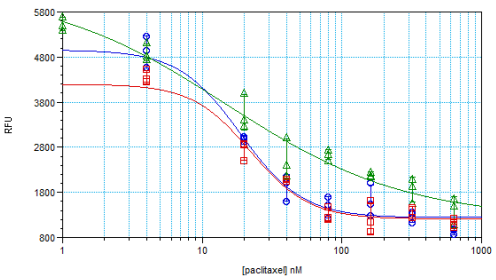
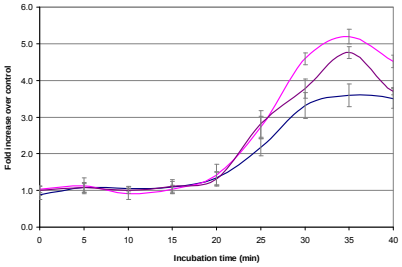
2. Kramer N, Walzl A, Unger C, Rosner M, Krupitza G, Hengstschläger M, Dolznig H. In vitro cell migration and invasion assays. *Mutat Res.* 752(1):10-24 (2013).
3. Zhang JH, Chung TD, Oldenburg KR. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen* 4(2): 67-73 (1999).
4. Partridge J, Flaherty P. An in vitro FluoroBlok tumor invasion assay. *J Vis Exp* 2009, pii: 1475.
5. Technical Bulletin #441 - Screening of Anti-Metastatic Compounds Using ZsGreen1 Reef Coral Fluorescent Protein (RCFP) Labeled HT-1080 Tumor Cells
6. Technical Bulletin #442 - Screening of Anti-Metastatic Compounds by a Fluorescence Based Tumor Cell Invasion Assay
7. Technical Bulletin #457 - Optimized Chemotaxis Conditions for Primary Blood Monocytes or THP-1 Cells Using Corning FluoroBlok 96-Multiwell Insert Plates
8. Application Note #484 - Migration of Human Mesenchymal Stem Cells using Corning FluoroBlok Cell Culture Inserts
9. Corning BioCoat™ Angiogenesis System: Endothelial Cell Migration Guidelines for Use

Figure 6. Reproducibility of Assays – Paclitaxel IC50s



HT-1080 cells in endpoint migration assay. IC50s calculated in three separate assays (n=3) were 15, 19, 22 nM; previously measured at 25 – 64 nM.[5-6]

Figure 7. Leukocyte Chemotaxis



THP-1 chemotaxis to 25 nM MCP-1. Peak response time in three separate assays (n=3) was 30-35 min.

Summary

Corning FluoroBlok cell culture inserts and insert systems yield results that are highly reproducible and correlate with previously published data[5-6]. There is a significantly increased fluorescence blocking range when compared to the original membrane.

This technology enables the use of a wide variety of cell types to study cell migration and invasion, including: immune cells, tumor cells and cell lines, stem cells[1,8], endothelial cells, and primary cells.

Advantages of Corning FluoroBlok inserts

+ true homogeneous assays – just add cells and chemoattractants
+ real-time, kinetic assays
+ suitable for screening, imaging and automation
+ multiplexing of fluorescent labels
+ easy to adapt assays from clear inserts
+ easily scalable from individual inserts to multiwells and HTS formats
+ standardization of protocols and assay conditions[4]
+ precoated options, *e.g.*, Corning BioCoat, offer time savings and consistent performance

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