An improved assay system for cell migration and invasion

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Abstract

In vitro cell migration and invasion assays are frequently used as model systems for quantifying the directed movement of cells towards a chemotactic 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 98% transmission of light from 360 - 690 nm as 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, monocyte chemotaxis to 25 nM MCP-1. Peak response time in three separate assays 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 Huntek, Inc. WorldView PRO. Membranes were immersed in commonly used biological solvents (water, saline, DMSCO in culture medium and 20% ethanol) for up to 24 hours and values were measured. Tumor Cell Migration, Invasion and IC50 Assay4-6

HT-1080 (human fibrosarcoma cell line) and 3T3 (mouse embryonic fibroblasts) cells were seeded at 25,000 cells/well. Chemotaxin was 5% FBS. For overnight kinetic assays, an Abbott Altralux plate was used to maintain a 5% CO2 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.

Endothelial Chemotaxis2

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.

Advantages of Corning FluoroBlok inserts

- easy to adapt assays from clear inserts
- 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 multiwell and HTS formats

References

5. Technical Bulletin #441 - Screening of Anti-Metastatic Compounds Using ZsGreen1 Reef Coral Fluorescent Protein (RFP) Labeled HT-1080 Tumor Cells
6. Technical Bulletin #442 - Screening of Anti-Metastatic Compounds by a Fluorescence Based Tumor Cell Invasion Assay
8. Application Note #484 - Migration of Human Mesenchymal Stem Cells using Corning FluoroBlok Cell Culture Inserts