Cell Migration and Invasion Quantification Assay with Acetic Acid-dependent Elution of Crystal Violet

Application Note

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Introduction

Cell migration is the movement of cells from one area to another, generally in response to a chemical signal, whereas cell invasion requires a cell to first penetrate an extracellular matrix (ECM) barrier by enzymatic degradation.

Corning's Transwell® permeable supports provide a relatively simple *in vitro* approach to performing cell invasion and migration assays. Transwells are convenient, ready-to-use permeable support devices prepackaged in multiwell plates. The unique, self-centered hanging design prevents medium wicking between the insert and the outer well.

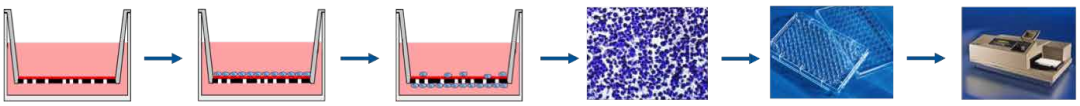
Corning Matrigel® matrix, a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, can be used as a barrier for invasion assays. The matrix includes laminin (major component), collagen IV, heparan sulfate proteoglycans, entactin/nidogen, and a number of growth factors and metalloproteases.

Crystal violet staining of cells is a widely used method for the evaluation of migration and invasion assays. To quantify migration/invasion, the conventional method is to manually count stained cells in certain fields under a microscope. However, this method is time-consuming, low-throughput, and subjective, which limits its application.

Here, we describe a method for quantifying cell migration and invasion with acetic acid-dependent elution of bound crystal violet. Crystal violet was eluted using 33% acetic acid and quantified by measuring the absorbance at 590 nm with a plate reader. This quantification method can be easily performed following conventional staining with crystal violet.

Materials and Methods

Workflow Overview



Cell Culture

HT-1080 (ATCC CCL-121), NIH/3T3 (ATCC CRL-1658) and MCF-7 (ATCC HTB-22) cells were purchased from American Type Culture Collection (ATCC). HT-1080 cells were cultured with MEM (Corning 10-009-CV) supplemented with 10% fetal bovine serum (FBS, Corning 35-076-CV). MCF-7 cells were cultured in MEM with 10% FBS and 0.01 mg/mL human recombinant insulin. NIH/3T3 cells were cultured in DMEM (Corning 10-013-CVR) with 10% FBS. Cells were cultured in Corning T-75 rectangular vented flasks (Corning) and supplied with fresh medium every 2 to 3 days. Cells were split after reaching 80% to 90% confluence.

Corning® Matrigel® Matrix Coating

Corning Matrigel basement membrane matrix (Corning 356234) was thawed on ice at 4°C overnight before use to ensure it thawed completely, but did not prematurely gel. All reagents, pipets, and containers that came in contact with the Matrigel matrix were pre-chilled prior to use. The Matrigel matrix was diluted in serum-free medium to a final concentration of 200 µg/mL and mixed thoroughly by gently pipetting the matrix up and down. Next, 100 µL of the diluted Matrigel matrix was carefully added to the center of each Transwell® insert (8 µm PET membrane, Corning 3464) for invasion assays. Transwell inserts meant for migration only were not coated with Matrigel matrix. The plate was incubated at 37°C for 1 hour to allow the Matrigel matrix to form a gel.

Cell Seeding

HT-1080, NIH/3T3, and MCF-7 cells were trypsinized and resuspended with culture medium without FBS. The cells were counted and diluted to a density of 5 x 10^5/mL with serum-free medium. Next, 150 µL of the cell suspension was seeded into the upper chamber of each Transwell. The final cell density was 7.5 x 10^4 cells/well. For the experimental group, 800 µL of culture medium with 10% FBS used as chemoattractant was added to the lower chambers. As a control, 800 µL medium without FBS was added to the lower chambers. The cells were cultured in a humidified incubator at 37°C with 5% CO_2 overnight.

Crystal Violet Staining

Transwell inserts were washed twice with phosphate-buffered saline (PBS, Corning 21-040-CV). The cells on the inside of the Transwell inserts were gently removed using moistened cotton swabs, and the cells on the lower surface of the membrane were then stained with crystal violet (Beyotime C0121) for 10 min. The Transwell inserts were washed twice with PBS to remove unbound crystal violet and then air-dried. The invaded and migrated cells were observed and imaged under a microscope.

Acetic Acid Elution and Quantification

Acetic acid (Sinopharm 10000208) was diluted to 33% (v/v) with ddH_2O. The bound crystal violet was eluted by adding 400 µL of 33% acetic acid into each insert and shaking for 10 min. The eluent from the lower chamber was transferred to a 96-well clear microplate (Corning 3599), and the absorbance at 590 nm was measured using a plate reader.

Standard Curve Generation

To generate a standard curve, the cells were diluted with culture medium to a series of cell densities and seeded into a 96-well clear microplate. Cells were cultured, stained, and quantified as described above. The standard curves of cell numbers versus absorbance were plotted.

Results and Discussion

Cell Lines with Varying Migration and Invasion Capacities

Invasion assays were validated with different cell lines. Invasive HT-1080 cells confirmed the invasion assay. Evaluation of low-invasive NIH/3T3 cells confirmed that the barrier inhibited invasion, and low-migratory MCF-7 cells were also tested as a control (Figure 1).

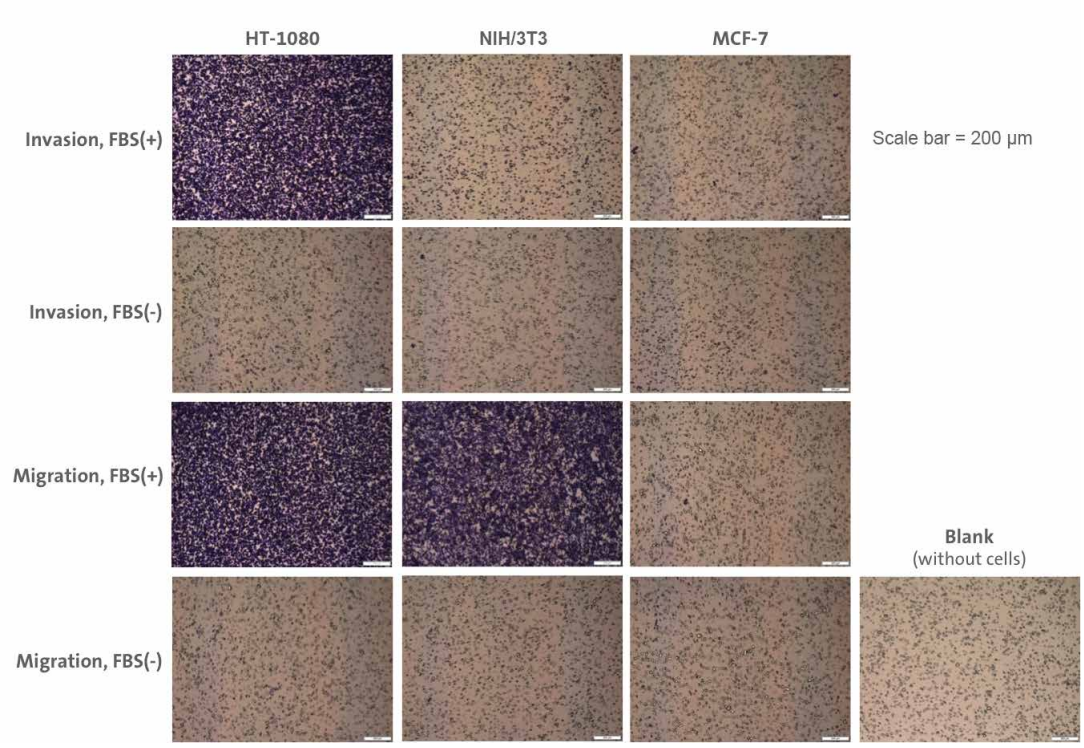


Figure 1. Invasion assays were validated with invasive HT-1080 cells, low-invasive NIH/3T3 cells, and low-migratory MCF-7 cells. The spots in the blank group are pores on the membrane.

Standard Curve

The absorbance values of HT-1080, NIH/3T3, and MCF-7 cells seeded at various cell densities were obtained by measuring the OD at 590 nm. The standard curves of cell numbers versus absorbance were plotted, and an equation for each cell line was generated (Figure 2). The R^2 values of these standard curves were greater than 0.99.

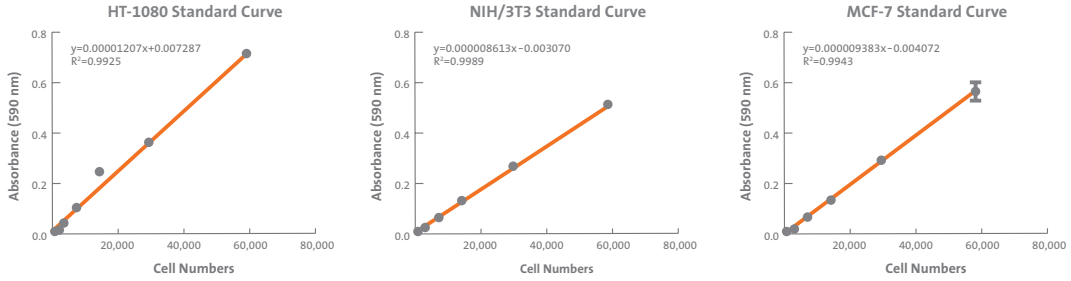


Figure 2. Standard curves of HT-1080, NIH/3T3, and MCF-7 cells.

Quantification of Migration and Invasion

Bound crystal violet in HT-1080, NIH/3T3, and MCF-7 cells was eluted using 33% acetic acid (Figure 3) and measured with a plate reader. Absorbance at 590 nm was subtracted by the data of blank Transwell® without cells. Invasion and migration assay results for HT-1080, NIH/3T3, and MCF-7 cells based on absorbance are shown in Figure 4.

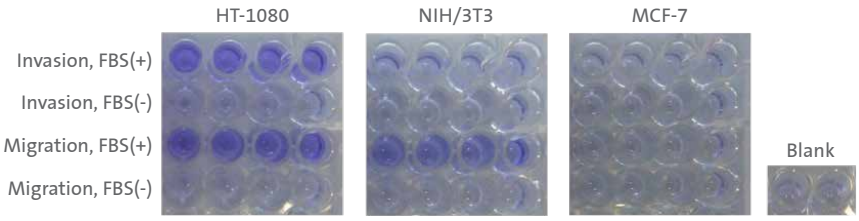


Figure 3. Eluted crystal violet in 96-well microplates.

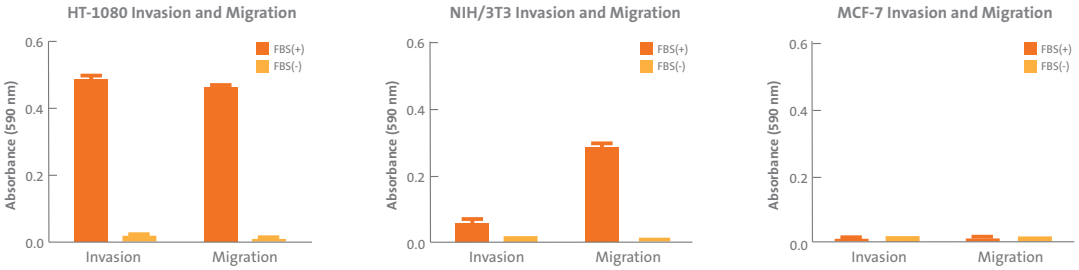


Figure 4. Invasion and migration assay results for HT-1080, NIH/3T3, and MCF-7 cells based on absorbance.

The total number of cells passing through the Transwell® membrane were determined by converting absorbance values to cell numbers using the equation from the standard curve and multiplying by the dilution factor. The percentages of invasion and migration were determined by dividing the number of invaded/migrated cells by the number of plated cells (Figure 5).

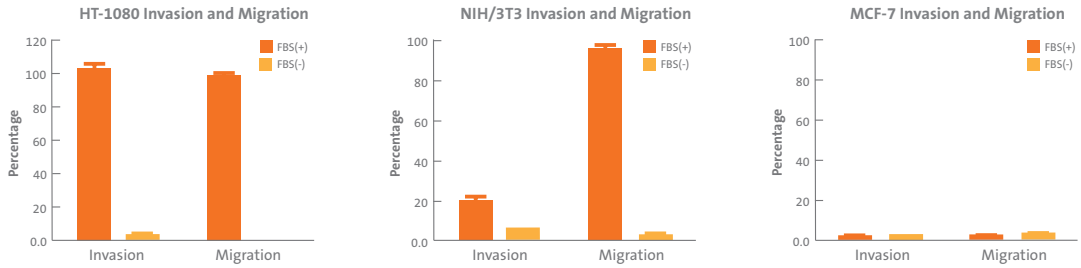


Figure 5. Percent invasion and migration of HT-1080, NIH/3T3, and MCF-7 cells.

Conclusions

Acetic acid elution of crystal violet can be easily performed following traditional staining with crystal violet by measuring the absorbance at 590 nm. This method is a practical and effective approach for quantifying cell migration and invasion assays that eliminates manual counting, which is time-consuming, labor-intensive, and subjective.

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