

Overview

High-content tumor cell migration assays in 3-dimensional extracellular matrix are a powerful tool for modeling and understanding the biology of this critical step in the process of metastasis. Currently available methods are not amenable to increased throughput required by studies of comparative pharmacology or small scale screening. We present here an automated approach to high-content tumor cell migration assays. A standard screening-sized plate with an array of embedded microchannels was designed and constructed from common thermoplastics. After filling the channels with 3D matrix, cells were placed at one end of the channel and migration into the channel was monitored via an imaging system. All liquid handling steps were performed by standard liquid handling robotics. Tumor cell migration in the channel was truly 3-dimensional. The information-rich data from these assays was used to rank the potency of migration inhibitors through 3D collagen, as well as gain additional insights into the compounds' activities related to cell proliferation and health. This approach is compatible with a variety of multiparametric, morphological and/or kinetic readouts.

An array of microchannels used to monitor tumor cell migration through 3D extracellular matrix

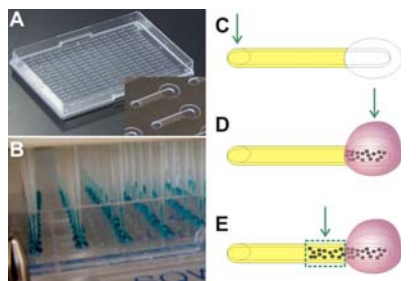


Figure 1. Device design and operation. A) An array of 192 microchannels in a microtiter plate format. Inset: close-up picture of individual microchannels. B) Interfacing liquid handling robotics with the microchannel plate. A CyBi®-Well (CyBio, AG) with a 25 μ L 96-tip head is shown here dispensing to the output port. C-E) Schematic of the basic steps in the 3D tumor cell migration assay. C) 800 nL extracellular matrix is added to the input port, filling just to the opposite end of the channel. D) Cells are added to the large port at the right end of the channel. E) Cells migrate into the matrix-filled channel over duration of the assay. Cells in the indicated dashed-line box are imaged and quantified.

Migration is collagen-dependent, 3-dimensional and correlates with metastatic potential

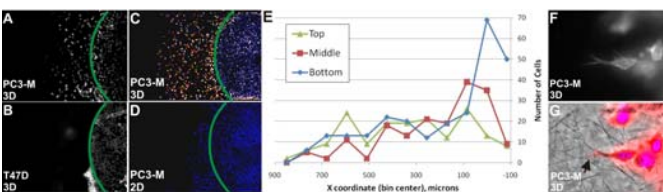


Figure 2. Tumor cell migration in type I collagen gel correlates with metastatic potential and is three dimensional. Metastatic PC3-M cells (A) or non-metastatic T47D cells (B) were plated in the output port of channels filled with 3D collagen. After 72 hours, cells were stained with Hoechst and imaged with a 4x objective. PC3-M cells were seeded in the output port of a channel filled with collagen (C) or a channel containing only media (D). After 5 days, cells were fixed and stained with DAPI and imaged under epifluorescence with a 10X objective at the channel bottom (0 μ m), middle of the channel (70 μ m), and channel top (140 μ m). Each image was colorized (blue, yellow, red respectively) and overlaid. The border between channel and output port is shown in green. E) Histogram of cell count relative to X-coordinate at different Z-heights in microchannel. Z-heights of PC3-M cells were binned as either top (140 μ m), middle (70 μ m) or bottom (0 μ m) based on the object intensities in the original stack of images used to create the overlay in figure 2C. (F-G) Invading PC3-M cells were fixed and stained with Alexa Fluor® 594-Phalloidin and DAPI. F) 20X fluorescent image of phalloidin staining. G) Overlay of phalloidin (red), DAPI (blue), and a phase contrast image revealing individual collagen fibers.

Kinetic Monitoring of Invasion

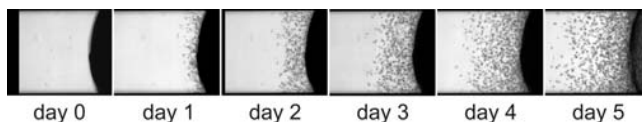


Figure 3. Tumor cell migration monitored over duration of assay. PC3-M cells migrated into type I collagen-filled microchannels over 5 days following the standard protocol. Phase contrast images were captured daily with a 4x objective.

Effect of inhibitors on migration

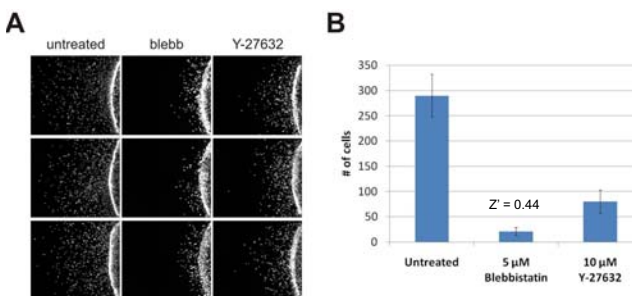


Figure 4. Quantitative analysis of inhibitors of migration. PC3-M cells migrated into type I collagen-filled microchannels over 5 days. Cells were treated with 5 μ M Blebbistatin, 10 μ M Y-27632, or an untreated solvent control. Growth media with inhibitors was replaced daily. Cells were fixed and stained with DAPI and imaged with a 4x objective. Images were automatically analyzed and cells quantified. A) Images of 3 replicates for each experimental condition. B) Average number of cells that had migrated into the matrix-filled channel. n = 16.

Multi-parametric analysis of inhibitor effects on invasion and cell health

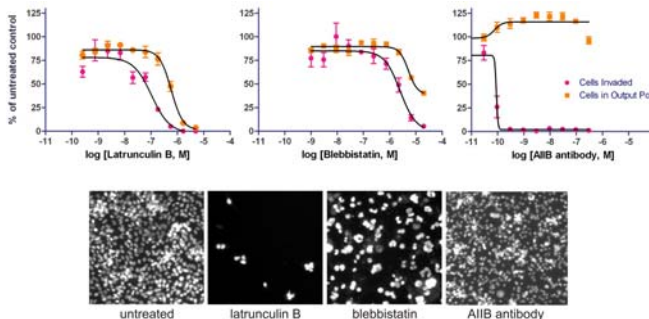


Figure 5. Profiling inhibitors of migration. Upper panel: PC3-M cells migrate into type I collagen-filled microchannels over 5 days. Media containing a titrated dose of inhibitor was replenished daily. The number of cells that migrated greater than 150 μ m was quantified and shown normalized the untreated control. Non-linear variable-slope curves were fit to the data (Graphpad Prism), n = 4. Lower panel: Images of Hoechst stained cells in the output port at the end of the assay.

Alternative modes of analysis

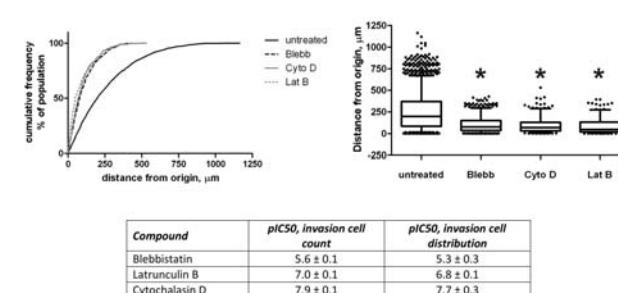


Figure 6. Migration analysis based on cell population distribution. PC3-M cells were treated with 6.7 μ M Blebbistatin, 0.56 μ M Latrunculin B, 7.6 nM Cytochalasin D or left untreated, for a 5-day migration assay in type I collagen. Upper left panel) The frequency of distances travelled by the cells is plotted cumulatively. For example, approximately 50% of the cells treated with Blebbistatin have not passed the origin line, and are located in the output port. Upper right panel) Box-whiskers plots for the distribution of distances travelled by the cells. The box indicates 25th, median, and 75th percentile, and whiskers indicate 5th and 95th percentile. Data points outside 5 and 95th percentiles are shown individually with dots. The distribution of cells treated with inhibitor was found to be significantly different from the untreated control with p<0.001, using the 1-tailed Mann-Whitney test. Table) IC50s and corresponding 95% confidence intervals were calculated based on number of cells invaded and also by distribution of 75th percentile of distance traveled. n=4. Strong concordance between the different methods of analysis was observed.

Migration through laminin-rich matrix demonstrates altered morphology

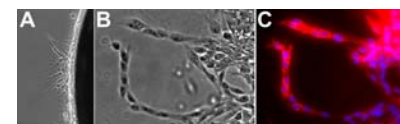


Figure 7. Invasion through laminin-rich extracellular matrix. Channels were filled with laminin-rich ECM (Matrigel™) with CyBi®-Well liquid handler. A) PC3-M cells initiating invasion into matrix one day after plating, as shown by 10X phase contrast microscopy. 5 days after plating, cells were fixed, permeabilized, and stained with Alexa Fluor® 594-Phalloidin and DAPI. B) Cells viewed with phase contrast microscopy. C) An overlay image of phalloidin (red) and DAPI (blue).

Conclusions

- These plates can be used for the quantitative assessment of tumor cell invasion through 3D collagen.
- The assay is fully automatable using liquid handling robotics and automated microscopy.
- Classes of compound action can be distinguished based on relative effects on invasion and cell health.
- In addition to the extent of invasion, a wide variety of information can be gleaned from this approach, including cell density, viability, and assessment of molecular markers such as phalloidin for probing F-actin morphology, as well as a full range of immunocytochemistry.
- The assay can be monitored kinetically.

Acknowledgements

Thanks to the lab of Raymond C. Bergan, MD at Northwestern University for kindly providing us with PC3-M cells and helpful discussions and insights.

Funding for this work was provided by NIH grant # 9 R44 CA133909-02 and # 5 R44 CA133909-03.

