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 plated 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 2D 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. Insert: close-up view of individual microchannels. B) Interface liquid handling robotics with the microchannel plate. C) CyBi®-Well (CyBio, AG) with a 25 μl 96-tip head is shown here dispensing into the output port. D) Schematic of the basic steps in the 3D tumor cell migration assay. E) 3D extracellular matrix is added to the input port, filling just to the opposite end of the channel. F) Cells are added to the large port at the right end of the channel. G) Cells migrate into the matrix-filled channel over duration of the assay. H) Chondrocytes in the media-filled channel show cell proliferation and health.

Migration is collagen dependent and 3-dimensional

Figure 2. Tumor cell migration in type I collagen gel is three dimensional. PC3-M cells were plated in the output port of empty channels (C,D) or matrix-filled channel over duration of the assay. Cells in the indicated dashed-line box are imaged and quantified.

Invasion can be monitored kinetically

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. 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.

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.
• Quantitative results consistent with compound profiling or small scale screening requirements can be obtained.
• 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 in kinetic mode.

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An automated high-content assay for tumor cell migration through 3-dimensional matrices

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Figure 5. Profiling inhibitors of migration. 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 μm was quantified and shown normalized the untreated control. Non-linear variable-slope curve were fit to the data. Corresponding IC50 values and 95% confidence intervals are shown in the table below.

n = 4.

Figure 6. Migration analysis based on cell population distribution, and assessment of final cell density. PC3-M cells were treated with 6.7 μM Blebbistatin, 2.16 μM Latrunculin B, 7.6 nM Cytochalasin D or left untreated, for a 5-day migration assay in type I collagen. A) 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. B) Box-whiskers plots for the distribution of distances travelled by the cells. The box indicates 25th, 50th, and 75th percentiles, and whisker indicates 10th and 90th percentiles. Data points outside the 10th and 90th 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 Kruskal-Wallis non-parametric test. For each condition, the distances migrated for all cells in four replicate channels were analyzed: 1,236, 1,422, 1,077, and 1,023 cells were analyzed for untreated, Blebbistatin, Latrunculin B, and Cytochalasin D respectively. In addition to cell migration, the density and viability of the PC3-M cells were evaluated in the output port. C) Dose-dependent effect on cell density in the output port. D) Live cells in the output port were stained with Hoechst 33342 (blue) and propidium iodide (red). Propidium iodide was taken up by cells with compromised membranes. 10x images are shown for the untreated control, 20μM Blebbistatin, and 7μM Latrunculin B.

Figure 7. Invasion through laminin-rich matrix results in altered morphology

Alternative and auxiliary modes of analysis

• Profiling inhibitors

- The assay can be monitored in kinetic mode.
- Quantitative results consistent with compound profiling or small scale screening requirements can be obtained.

- 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 in kinetic mode.