Abstract

Cell migration, the relocation of cells, is relevant to wound healing, immunology, embryonic development, and irregular cellular events such as cancer metastasis. Cell migration assays are used to measure cell motility in a controlled environment and are frequently prepared and analyzed manually. In this project, we utilized an adapted cell migration assay that incorporates a circular, dissolvable biocompatible gel in each well of a microwell plate to determine if automatic cellular imaging can effectively image and analyze cell migration. We studied the migration of two different cancer cell lines: HT1080 (derived from human fibrosarcoma cells) and U2OS (derived from a human osteosarcoma) that were plated at an optimized density to generate a confluent monolayer in a 384-well microplate containing the circular biocompatible gel. Five chemotherapeutic compounds that inhibit cell migration were assessed at various concentrations, and the effects on cell motility over 45 hours duration were measured. A time-lapse series of images was collected so that the closure of the cell-free area could be measured over time. Image analysis software was used to quantify the area of the well covered by the cells. Results of analysis using either transmitted light images or fluorescent images were compared. The results indicate that Colchicine, Cytochalasin D, and Nocodazole inhibit cell migration at specific concentrations. Lastly, these experiments demonstrate that automated microscopy can be used to effectively image and analyze cell migration assays in transmitted light and fluorescence.

Cell Migration Analysis Using Automated Cell Imaging

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INTRODUCTION

Cell migration is essential in many biological processes such as embryonic development, immunological responses, wound healing, and metastasis of cancer cells. In the activation of the innate immune system, neutrophils, macrophages, eosinophils, and other immune cells migrate to the site of inflammation by responding to cytokine signals and chemokine gradients. In most cases this is beneficial, as clearing of damaged tissue is very important, as is regeneration of tissue, which is prompted by the cytokine secretions of M2 macrophages (Shiraishi et al., 2016; Julier et al., 2017). However, in many auto-immune diseases, including rheumatoid arthritis, migration and accumulation of immune cells can have disastrous effects (Nevius et al., 2016). Additionally, cell motility, which is upregulated in the tumor microenvironment of individuals affected by cancer, exacerbates cancer progression (Barret et al., 2017). Thus, migration of cells, and the measurement of this motility is an area of interest when studying pathogenesis of disease and development of potential therapies and drug candidates.

Cell migration assays are used to measure cell motility in a controlled environment. Frequently, “scratch” assays are utilized for this purpose. After cultivating cells to confluency in a microwell plate, a pipet tip is typically used to make a thin scratch, or wound, in each well. Over time, cells migrate into the wounded area. While this assay is affordable, the wounds are often not identical in size or location in the well, the manual preparation of scratches is laborious and time-consuming, and it is not amenable to screening in a 384 well microplate format. To improve both throughput and reproducibility, Platypus Technologies adapted this assay to a microwell plate that incorporates a circular, dissolvable biocompatible gel (384 well Oris Pro plate). The circular gel creates uniform cell-free zones in the middle of each well, and dissolves spontaneously one hour after plating the cells. This assay enables reproducibility and high throughput for cell-migration experiments and is compatible with automated imaging systems.

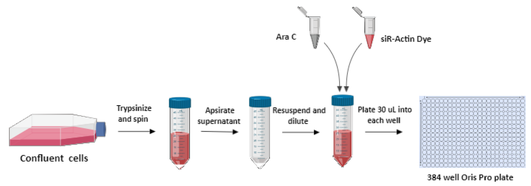
In this project, cell migration of fibrosarcoma and osteosarcoma cell lines that were plated in 384 well Oris Pro plates was imaged and analyzed using the ImageXpress® Pico Automated Cell Imaging System and CellReporterXpress™ Automated Imaging and Analysis Software. The purpose of this project was to demonstrate that reproducible cell migration assays can be completed using automated microscopy. Additionally, chemotherapeutic compounds, including Cytochalasin D, Colchicine, and Nocodazole were used to treat wells at optimized concentrations with the aim of inhibiting cell migration. We hypothesized that the chemotherapeutic compounds would inhibit cell migration and that automated microscopy could be used to successfully image and analyze cell motility. Here, we show that Cytochalasin D, Colchicine, and Nocodazole significantly inhibit cell migration at the concentrations used and that cell migration imaging and analysis can be performed using automated microscopy.

MATERIALS

- **Assay Reagents and Cells**
  - HT 1080 fibrosarcoma cell line (ATCC P/N CCL-121)
  - U2 OS bone cancer cell line (Millipore Sigma P/N CLL1037)
  - SiR-Actin Kit (Cytoskeleton Inc. P/N CY-SC001)
  - Cytosine β-D-arabinofuranoside hydrochloride (Ara C) (Sigma Aldrich P/N C1768)
  - Oris™ Pro Cell Migration Assay (Platypus Technologies P/N PRO384CMA1)
- **Automated Imaging**
  - ImageXpress Pico Automated Cell Imaging System with CellReporterXpress software (Molecular Devices, LLC)

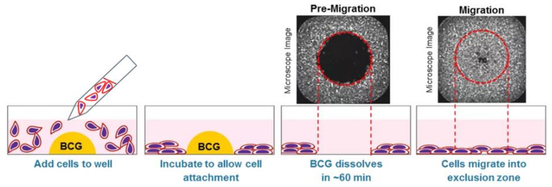
METHODS

Plating of Cells that have been Treated with Fluorescent Dye and Cell Inhibitor



After cells were diluted in media to their working concentration, Cytosine β-D-arabinofuranoside hydrochloride (Ara C), a known inhibitor of cell division, and SiR- Actin, a fluorescent dye that has been shown to harmlessly stain the actin of live cells, were added to a final concentration of 20 µM and 0.1 µM, respectively. The purpose of using a cell inhibitor was to ensure that cell migration was being detected as opposed to cell replication. After cells were treated with cell inhibitor and actin dye, they were plated in 384 well Oris Pro Cell Migration plates at 10,000 cells/well at a final volume of 30 µl.

Oris Pro Cell Migration Assay



Treatment of Plated Cells with Chemotherapeutic Compounds

After cells had attached and gel had dissolved for 2 hours, 30 µl of each compound was added to the 30 µl of cells already in the wells. Cells were returned to incubator for 1 hour before imaging of the plate commenced.

	Cont.	Isoproterenol		Colchicine		Nocodazole		Cytochalasin D		AraC	Cont.	
	13	14	15	16	17	18	19	20	21	22	23	
A	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	HT 1080
B	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
C	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
D	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
E	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
F	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	U2 OS
G	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
H	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
I	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	
J	0	10 uM	2.5 uM	150 nM	37 nM	1 uM	250 nM	150 nM	37.5 nM	5 uM	0	

Table 1: The compound concentrations used to test cell migration inhibition are shown. The concentrations utilized were based on prior experiments that measured the ability of these compounds to inhibit cell migration without inducing cytotoxic effects.

Imaging System

Parameter	Setting
Imaging System	ImageXpress Pico system
Read mode	Transmitted Light and/or Cy5 Fluorescence
Read type	Timelapse (discontinuous intervals)
Time Points (hr post-compound)	1, 2, 4, 6, 21, 24, 29, 45
Magnification	4X
Number of sites imaged	1
Wavelengths/Filters	Transmitted light: <5 ms exposure Cy5/ siR-Actin: 500-1000 ms
Image analysis	Transmitted Light, Large Cells Cell Scoring – Cy5 nuclei & cytoplasm

Table 2: A discontinuous time-lapse series was collected over a 45-hour period using the settings shown here. Image analysis measured cell migration by calculating the area covered by cells in each well of every treatment group and control group at each time point. The analyses selected were previously optimized for both Transmitted light and fluorescent analysis.

RESULTS

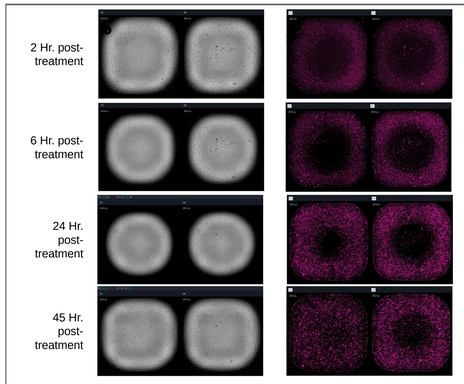


Figure 1. Example Transmitted Light and SiR-Actin images of an untreated HT1080 cell control well (left) compared to a well treated with 1 µM Nocodazole (right) at 4 representative timepoints.

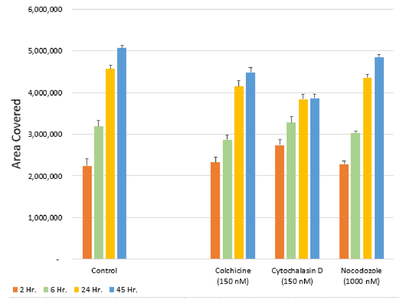


Figure 2. Analysis of fluorescently labeled cells over time demonstrates that compound-treated wells exhibited decreased cell migration.

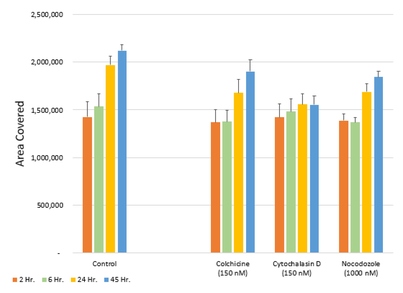


Figure 3. Transmitted Light analysis over time demonstrates that compound-treated wells exhibited decreased cell migration.

RESULTS

Alpha level = 0.05				
		U2OS	HT 1080	
		TL	TL	Cy5
Compound	Concentration (nM)	Significant?	Significant?	Significant?
Colchicine	150	yes	yes	yes
Cytochalasin D	150	yes	yes	yes
Nocodazole	1000	yes	yes	yes

Table 3. The two tailed t test reveals the above compounds significantly affected cell migration of HT 1080 and U20S in all analyses that were run using the analysis protocols in CellReporterXpress software.

CONCLUSION

- At the concentrations administered, Colchicine, Cytochalasin D, and Nocodazole significantly decrease cell migration.
- The agreement between the Transmitted light and fluorescent analyses indicate that cell migration assays may be run with or without fluorescent SiR-Actin labeling in cell lines that retain the dye.
- With the use of the OrisPro 384 well migration assay, medium throughput screening for inhibitors of cell migration may be performed.
- Timelapse assays may be conveniently imaged and analyzed with ImageXpress Pico Automated Imaging System and CellReporterXpress software.

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