A novel HTS-adaptable assay for cell migration of hESC-derived progenitors

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Introduction

Cell migration is important for many normal and pathological developmental and regenerative processes, such as wound repair or cancer metastases. The identification of cell migration modulators can be a useful first step in the discovery of therapeutics that aid or block those processes. To that end, we are developing an HTS-amenable assay for identifying cell migration modulators using human embryonic stem cell (hESC) derived hNP1™ neural and hMPro™mesenchymal progenitor cell lines (ArunA Biomedical) and a novel 96-well based cell migration assay platform (Oris™ Cell Migration Assay; Platypus Technologies). With the Oris™ platform, cells are plated on the substratum of choice around stoppers used to create central exclusion zones. Once the stoppers are removed, cells migrate into the exclusion zone and are then stained for detection using fluorescent plate readers and/or microscopy. Here, we found that cytochalasin D, a disruptor of actin filaments, inhibits hNP1™ and hMPro™ migration. Also, we show that certain neurotrophic factors have chemokinetic effects on hNP1™ cells. Together, these data demonstrate that this system can readily identify modulators of cell migration, including growth factors, small molecules, extracellular matrix extracts and components, and artificial substrata.

Experimental Design

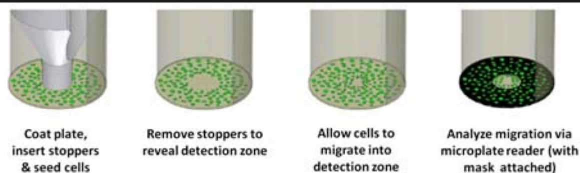

Figure 1. Schematic of the Oris™ Cell Migration Assay

- hNP1™ were plated at 60,000 cells per well onto Matrigel-coated Oris™ Assay plates in proliferation medium (PRO; AB2, ANS, LIF and bFGF) for ~16 hrs at 37°C.
- hMPro™ were plated at 40,000 cells per well onto uncoated Oris™ Assay plates in complete medium (αMEM plus 10% FBS) for ~16 hrs at 37°C.
- Stoppers were then removed, except in the "no migration" control column (STOPPER). The plating medium was replaced with test medium and cells were then incubated at 37°C for either 36 hrs (hMPro™) or 72 hrs (hNP1™) to allow for migration.
- At the end of the migration period, stoppers removed from "no migration" control wells and all cells were stained at 37°C for 30-60 minutes with calcein-AM (5 μg/mL).
- Plates were read using a Flexstation3 microplate reader (ex 494 nm/ em 517 nm) and then imaged by epifluorescence microscopy.

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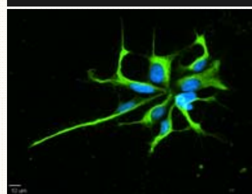
STEMEZ™ hNP1™



Marker	%positive
Nestin	>90
Musashi1	>90
SOX2	>90
Oct4	<5

Figure 2. STEMEZ™ hNP1™ neural progenitors.

hNP1™ cells were originally derived from the H9 (WA09) human embryonic stem cell line using defined, serum-free conditions (e.g., Shin, et al., 2006), proliferate as adherent monolayers and maintain a stable karyotype for multiple (>10) passages, making them scalable for HTS format (96- or 384-well) assays. hNP1™ express proneural markers (nestin, green; also see table) and are capable of differentiation into multiple neuronal phenotypes upon withdrawal of bFGF.

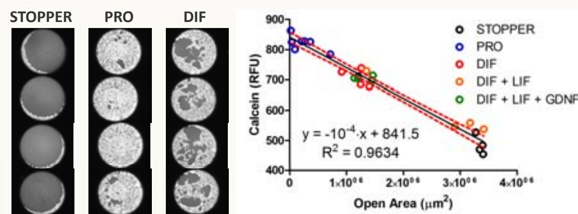


Figure 3. Use of calcein to measure hNP1™ cell migration on a microplate reader.
To develop a homogenous HTS-amenable assay, we tested the cytoplasmic dye calcein as a surrogate for time- and computationally-intensive area measurements of detection zone coverage by hNP1™ cells. Cells were incubated for 72 hrs in proliferation (PRO) or differentiation (DIF) media with and without LIF and GDNF. Calcein fluorescence correlated well with area measurements obtained using ImageJ.

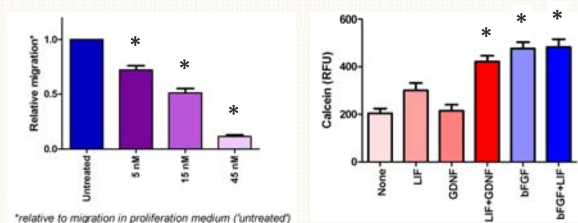
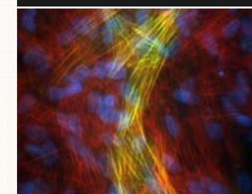


Figure 4. Inhibition and stimulation of hNP1™ cell migration. *Left*, Cytochalasin D inhibited hNP1™ migration in a concentration dependent manner with an IC_{50} of ~15 nM, indicating that inhibitors of migration can be readily detected. *Right*, Preliminary results indicate that bFGF alone stimulates hNP1™ migration (*, P < 0.05 when compared to no growth factor control). In contrast, the combination of LIF and GDNF has a synergistic chemokinetic effect (*, P < 0.05).

STEMEZ™ hMPro™



Marker	%positive
CD73	>90
CD90	>90
CD105	>90
Oct4	<5

Figure 5. STEMEZ™ hMPro™ mesenchymal progenitors.

Confocal image of STEMEZ™ hMPro™ cells stained for the early smooth muscle marker (αSMA; green), F-actin (red) and nuclei (blue). Exposure to 10ng/mL of transforming growth factor beta 1 (TGF-β1) for 12 days induces expression of alpha smooth muscle actin (αSMA) in hMPro™ mesenchymal progenitor cells, suggesting their ability to differentiate along the smooth muscle lineage. hMPro™ cells also express mesenchymal progenitor markers (see table).

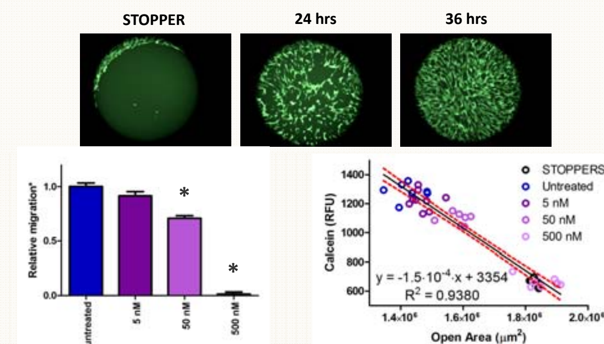


Figure 6. Inhibition of hMPro™ cell migration.
Top, hMPro™ cell migration covering the detection zone occurs over the course of 36 hours.
Bottom left, Cytochalasin D inhibits hMPro™ migration in a dose-dependent manner.
Bottom right, Calcein fluorescence correlates well with open area measurements, as seen in Figure 3.

Conclusions

- Using hNP1™ neural and hMPro™ mesenchymal progenitors, the Oris™ Cell Migration Assay can quantitatively detect both stimulators and inhibitors of cell migration and has the potential for adaptation as a homogenous HTS-amenable cell-based assay.