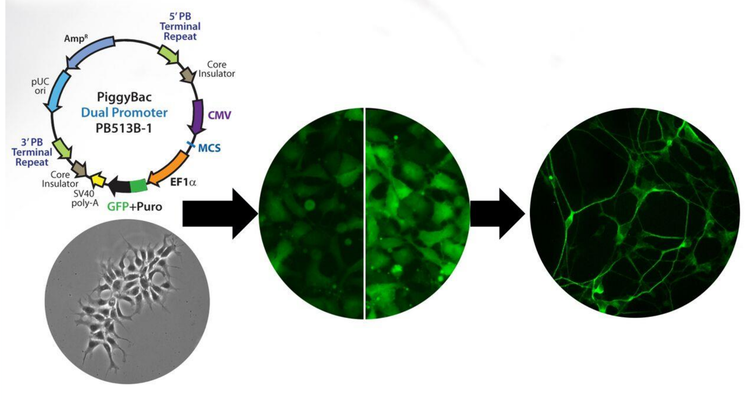
GFP+ HUMAN STEM CELL DERIVED NEURONS AMENABLE TO HIGH CONTENT NEURITE OUTGROWTH AND HIGH THROUGHPUT TOXICOLOGY ASSAYS

Tseten Yeshi[3], Jamie Chilton[2], Anirban Majumder[2], Jack Crawford[3], Jane Le[2] and Steven L. Stice[1].
[1]Regenerative Bioscience Center, University of Georgia, Athens, GA, USA, [2]ArunA Biomedical, Inc., Athens, GA, USA, [3]Hera Biolabs, Inc. Lexington, KY, USA

INTRODUCTION

High throughput (HTS) and high content screening (HCS) assays often utilize primary or stem cell sources, which are not amenable to large-scale screening and can require extensive cell culture and processing prior to imaging. Our goal was to develop GFP+ human stem cell derived neural progenitors to provide a scalable solution to the previously labor intensive, single-end-point, and hence limited nature of discovery and toxicology assays, while preserving the breadth and quality of data. Human neural progenitors derived from embryonic stem cell (WiCell h9) were modified with a non-viral vector encoding a Green Fluorescent Protein (GFP) reporter gene using a selectable *piggyBac™* system to produce the ArunA/Hera genetically modified hNP1GFP+™ line. Upon differentiation and selection, MAP2 expression increased to >90%, resulting in more mature neurons that exhibit significantly longer neurite length post-thaw. A cytotoxicity profile was generated for the hNP1GFP+™ human neural progenitors to ensure response to toxins, and detection and quantification under assay conditions. Cell viability and cell migration, two established neurotoxicity endpoints, were measured in cultures exposed to a panel of five compounds. A CellTiter96™ viability assay and a high throughput Cell Migration/Proliferation assay were used. hNP1GFP+™ cells exhibit detectable dose dependent response to toxins tested, (cytotoxicity and cell migration) providing a sensitive cell based human neurotox assay platform. Positive and negative controls produced results as expected, and can thus be used to normalize multi-plate data in larger screens. Overall, we conclude that hNP1™ Human Neural Progenitor Cells can be genetically modified and expanded to produce HCS and HTS assay ready cells.

Producing GFP+ Neurons from Non-Transduced hNP1™



RESULTS

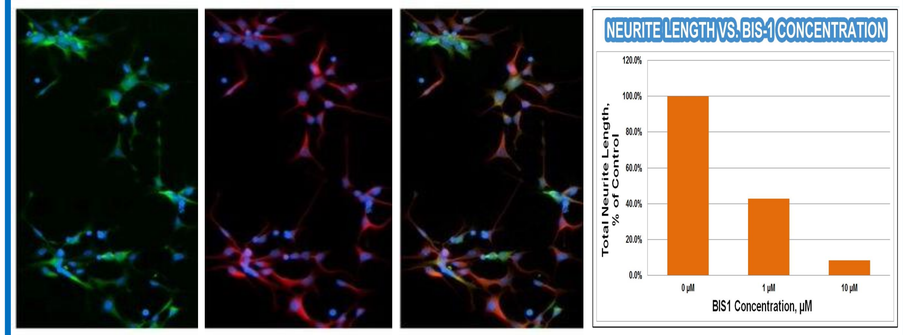


Figure 1. Characterization of GFP+ Neurons Derived from Transfected hNP1™ Cells. Puromycin-selected GFP+ hNP1 cells were differentiated into neurons that are 96% positive for MAP2 and cryopreserved. These images were taken after cells were fixed and stained 24 hours post-thaw. All three images are cross sections of one field. A) Neurons expressing GFP (green) and stained for DAPI (blue). B) Stained for MAP2 (red) and DAPI. C) Combined imaged of red, green, and blue channels. These cells were then successfully used in a neurite outgrowth toxicity assay using bisindolylmaleimide 1 (BIS1).

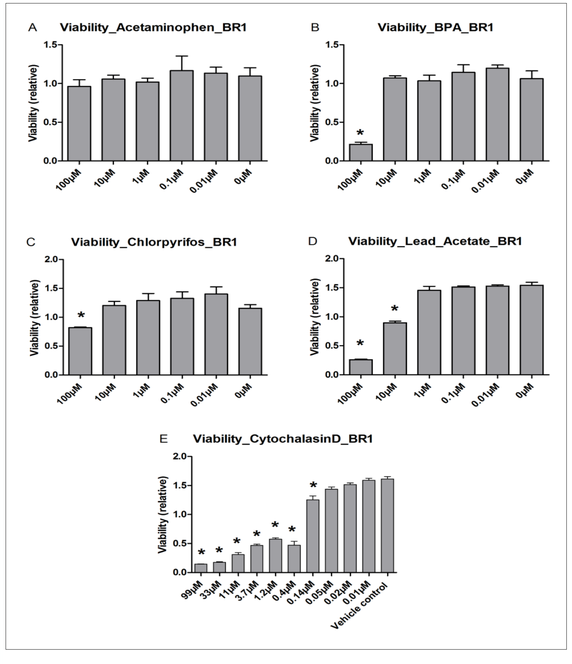


Fig 2. Screening Platform 1 – Cytotoxicity

hNP1GFP+ cells were incubated in test compounds for 24 hours in a 96-well assay plate. Post incubation, cell viability was assayed using CellTiter96 assay reagent. Changes in fluorescence, proportional to the number of live cells in a well, were quantified using a plate reader (FlexStation3). The chart above presents relative viability on the vertical axis. Viability at each dose was compared to that for a DMSO (vehicle) only control on the plate. Significant reduction in viability is noted with a * ($p<.05$) and error bars represent SEM. A) Acetaminophen was chosen as negative control, E) Cytochalasin-D was chosen as a positive control.

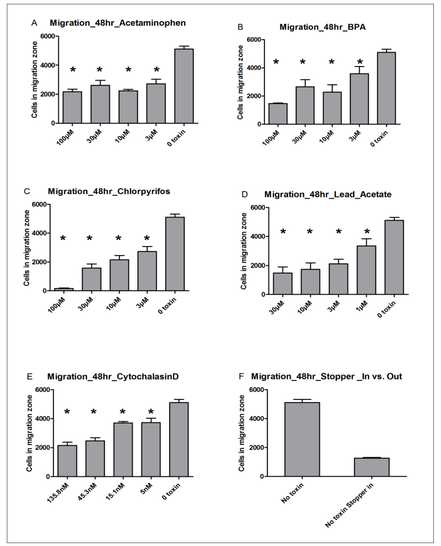


Figure 3. Screening Platform 2 – Cell migration

hNP1GFP+™ cells were incubated and exposed to test compounds for 48 hours in a 96-well ORIS cell migration assay plate. Cell migration was quantified using high content imaging on a Cellomics ArrayScan VTI imager. The charts represent the number of cells within the migration zone on the vertical axis. Migration at each dose was compared to a DMSO (vehicle) only control on the plate. Significant reduction in migration is noted with a * ($p<.05$) and error bars represent SEM. A) Acetaminophen was chosen as negative control. We observe a significant reduction in migration with Acetaminophen over no treatment control, although further dose-dependent decrease in migration was not apparent. E) Cytochalasin-D was chosen as positive control. F) Compares migration in no treatment control with culture wells where silicone stoppers physically prevent cells from migrating. B,C,D) Test compounds show a dose dependent trend.

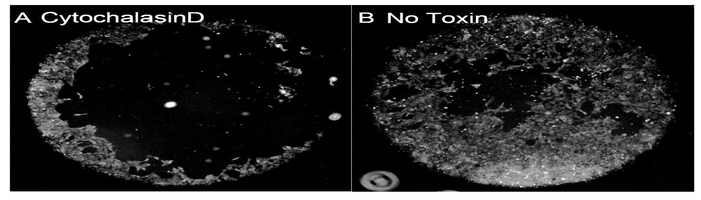


Figure 4. Images of hNP1GFP+™ cells in the migration zone of a 96-well ORIS migration assay plate acquired using a GFP filter set after 48 hrs. incubation with test compounds. Inhibition of cell migration by Cytochalasin-D, an established inhibitor of cell migration, is apparent in A compared to migration in absence of an inhibitor in B (DMSO only). The observed differences are consistent with quantified differences presented in **Fig 3**.

SUMMARY

By eliminating the need for fixing and staining cells, these GFP+ human neurons provide a scalable means to analyze neurite outgrowth in live cells spanning the course of hours to days following exposure to test compounds.

hNP1GFP+ cells differentiate with high efficiency into mature neurons allowing for Neurotoxicity assays in a high throughput manner.

Human stem-cell derived neurons show better sensitivity to neural toxins than mouse cortical neurons in a neurite outgrowth HCS assay, providing a more accurate toxicology model. (Harrill JA, et al.)