Video Article

High Throughput Characterization of Adult Stem Cells Engineered for Delivery of Therapeutic Factors for Neuroprotective Strategies

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Abstract

Mesenchymal stem cells (MSCs) derived from bone marrow are a powerful cellular resource and have been used in numerous studies as potential candidates to develop strategies for treating a variety of diseases. The purpose of this study was to develop and characterize MSCs as cellular vehicles engineered for delivery of therapeutic factors as part of a neuroprotective strategy for rescuing the damaged or diseased nervous system. In this study we used mouse MSCs that were genetically modified using lentiviral vectors, which encoded brain-derived neurotrophic factor (BDNF) or glial cell-derived neurotrophic factor (GDNF), together with green fluorescent protein (GFP).

Before proceeding with *in vivo* transplant studies it was important to characterize the engineered cells to determine whether or not the genetic modification altered aspects of normal cell behavior. Different culture substrates were examined for their ability to support cell adhesion, proliferation, survival, and cell migration of the four subpopulations of engineered MSCs. High content screening (HCS) was conducted and image analysis performed.

Substrates examined included: poly-L-lysine, fibronectin, collagen type I, laminin, entactin-collagen IV-laminin (ECL). Ki67 immunolabeling was used to investigate cell proliferation and Propidium Iodide staining was used to investigate cell viability. Time-lapse imaging was conducted using a transmitted light/environmental chamber system on the high content screening system.

Our results demonstrated that the different subpopulations of the genetically modified MSCs displayed similar behaviors that were in general comparable to that of the original, non-modified MSCs. The influence of different culture substrates on cell growth and cell migration was not dramatically different between groups comparing the different MSC subtypes, as well as culture substrates.

This study provides an experimental strategy to rapidly characterize engineered stem cells and their behaviors before their application in long-term *in vivo* transplant studies for nervous system rescue and repair.

Video Link

The video component of this article can be found at https://www.jove.com/video/52242/

Introduction

A major issue with implementing useful therapies for treatment of nervous system disorders is in developing effective methods that prevent further degeneration and also facilitate recovery of function. An innovative strategy is to genetically engineer stem cells *ex vivo*, for production of neuroprotective factors, prior to their transplantation. This combination of cell-based therapy, coupled with a type of gene therapy, provides a powerful method for the treatment of disease or injury-induced neuronal death in the nervous system.

Neurotrophic factors are essential for growth and survival of developing neurons as well as maintenance and plasticity of mature neurons. A number of studies have demonstrated significant roles of neurotrophic factors in promoting initial growth and differentiation of neurons in the central and peripheral nervous system (CNS and PNS) and they can also stimulate regeneration *in vitro* and in animal models of neural injury[1]. Brain-derived neurotrophic factor (BDNF) is highly expressed in the CNS and plays important roles in regulating neural development, synaptic plasticity and repair[2]. Glial cell line-derived neurotrophic factor (GDNF) promotes survival of many types of neurons including dopaminergic and motorneurons[3]. Thus, an important strategy for neural repair is to provide exogenous sources of neurotrophic factors to the injured or diseased regions of the nervous system.

Multipotent bone marrow-derived mesenchymal stem cells (MSCs) hold great potential for delivery of therapeutic proteins to treat the damaged or diseased nervous system. Transplantation of MSCs has attracted considerable attention in efforts to develop patient compatible cell-based therapies since they have a number of advantages including, 1) relative ease of isolation and maintenance, 2) multipotential capacity, 3) little ethical concerns, 4) ability to survive and migrate following transplantation and 5) potential for autologous transplantation[4,5]. Promising results have been reported with use of naïve and genetically engineered MSCs in animal models for a number of different neurodegenerative conditions, including spinal cord injury[6,7], stroke[8,9], myelin deficiency[10], and retinal degeneration[11-13]. Coupling cell transplantation with delivery of neurotrophic factors from genetically engineered stem cells is a novel and important neural repair strategy.

An essential step in developing cell-based therapeutic factor delivery systems is to determine the normal health of the engineered cells. As such, the principal purpose of this study was to evaluate general growth parameters of genetically engineered adult stem cells. An important approach to rapidly assess multiple cell parameters is to employ cellular image-based high-through screening (HTS), often referred to as high content screening (HCS) procedures[14]. This technology allows automated image acquisition and analysis and this approach is particularly well suited for stem cell research applications. In this project we developed a profiling platform that allows for the rapid characterization and optimization of cell substrate preferences and cellular functions with genetically engineered adult stem cells employing a HCS system.

Protocol

1. Substrate Preparation for 96-well Plates

1. Create a map of the 96-well plate outlining the different substrates and cell-types to be examined (**Figure 1**).
2. Obtain the stock solutions of different substrates [poly-L-lysine, fibronectin, collagen type I, laminin, and entactin-collagen IV-laminin (ECL)], a 96-well multiwell plate and prepare a work station in a sterile cell culture hood.
3. Prepare individual substrates by diluting stock in sterile phosphate buffered saline (PBS) to a final concentration of 5 µg/ml (this concentration was previously determined based on a substrate concentration-dependent assay for growth and proliferation of cells). Mix using a vortex before pouring into a sterile reservoir.
4. Add 100 µl of substrate solution into each well according to the 96-well map (**Figure 1**) (a 12- or 8-channel micropipette is convenient for micropipetting into a 96-well plate). Seal the lid to the 96-well plate using a strip of Parafilm and store overnight at 4 °C.

2. Cell Plating and Time-lapse Imaging

NOTE: Mouse MSCs were isolated from the bone marrow of adult C57BL/6 mice and maintained as an adherent cell line. MSCs were infected using lentiviral vectors to engineer them to secrete brain-derived neurotrophic factor (BDNF; human cDNA) and glial cell-derived neurotrophic factor (GDNF; human cDNA) using lentiviral vector's encoding BDNF (LV-BDNF; CMV-BDNF-IRES-GFP), GDNF (LV-GDNF; CMV-GDNF-IRES-GFP), and green fluorescent protein (GFP, LV-GFP; CMV-GFP).
NOTE: Culture media for mouse mesenchymal stem cells (MSCs) is Iscove's Modified Dulbecco's Medium containing 10% hybridoma-qualified fetal bovine serum, 10% equine serum, 2 mM L-glutamine, and 10,000 U/ml penicillin, 10 mg/ml streptomycin. The five different types of mouse MSCs (MSCs, GFP-MSCs, BDNF-GFP-MSCs, GDNF-GFP-MSCs and BDNF/GDNF-GFP-MSCs) were plated at about 30% confluence in T75 cell culture flasks.

1. **Cell Plating**
   1. On the following day, remove the substrate solutions by aspiration and rinse each well with approximately 200 µl of sterile PBS, twice. Add cell culture media (200 µl/well) to each well after the final PBS rinse. Place the 96-well plate into a cell culture incubator set at 37 °C and 5% CO_2 for equilibration.
   2. While the 96-well plate is equilibrating in the incubator, harvest the cells (MSCs in T75 flasks should be approximately 70% confluent at time of harvesting/plating) by collecting the growth media (referred to as conditioned media) from the T75 flask and storing in a 15 ml conical tube under sterile conditions (this conditioned media will be used in step 2.1.4 below).
   3. Add 8 ml of sterile PBS to the flask and gently swirl and then pipette off the PBS and add 1 ml of 0.05% trypsin and 0.01% EDTA solution to detach the cells from the culture surface of the T75 flask. Monitor cell detachment by viewing the flask using an inverted microscope equipped with phase contrast optics.
   4. When the cells have detached, immediately add 8 ml of the conditioned media (collected in step 2.1.2) to the flask. Collect the cell suspension and transfer to a 15 ml conical centrifuge tube and centrifuge for 4 min at 450 x g to pellet the cells.
   5. Remove the supernatant and resuspend the cell pellet in 200 µl of fresh and warmed (37 °C) cell culture media.
   6. Determine the number of cells in the cell suspension by performing a trypan blue viable cell count using a hemocytometer. Plate the cells at a density of approximately 300 cells/well into the appropriate wells of the 96-well plate.
   7. Repeat these steps for each population of cells.

2. **Time-lapse Imaging**
   1. Once all of the MSCs have been plated, place the 96-well plate into an incubator for 2 hr to allow the MSCs to attach to the substrate.
   2. Start the HCS system and wait for 2 hr for the system to equilibrate. Set the environmental controller to 37 °C and connect a mixed gas cylinder containing 5% CO_2 in air to the HCS system environmental chamber supplying a constant air source.
   3. Remove the 96-well plate from the incubator following the 2 hr equilibration period and place directly into the cell growth chamber of the HCS system. Allow 30 min for equilibration to account for any heat-related expansion of the plate and then start the image acquisition and analysis software to configure the plate settings.
   4. Select the 20X objective for imaging. Select two wells per condition [*i.e.,* GFP-MSCs on Fibronectin *etc.* (6 substrates x 5 MSC subtypes for a total of 30 conditions x 2 replicates = 60 wells in total)] for setting up time-lapse imaging. Choose two sites for imaging within each well.
   5. Choose the correct light wavelengths for imaging.
      NOTE: Two different wavelengths (Phase contrast and GFP fluorescence) were selected for time-lapse imaging.

6. Focus on the well bottom using laser autofocus and take test images for multiple sites and multiple wells to find an optimized focal plane.
7. Once the focus has been established, begin capturing images every 5 min for 48 hr for all 60 wells (120 sites).
8. Feed the cells every 24 hr by removing the 96-well plate from the HCS system. Remove 75 µl of media from each well and add 100 µl of fresh media to each well (equilibrate this fresh media at 37 °C and 5% CO_2).
9. At the end of the time-lapse experiment, remove the 96-well plate from the HCS system. Under sterile conditions, collect the conditioned media samples from each well and transfer these samples to another 96-well plate.
   NOTE: These samples can be used for further analysis by performing ELISA for neurotrophic factors.
10. Prepare the 96-well plate with cultured MSCs for additional assays such as Ki67 cell proliferation assay or propidium iodide live/dead staining assay (see details below).
11. Perform time-lapse imaging analysis for cell migration/cell tracking as described in **section 5** below.

3. Ki67 Cell Proliferation and Propidium Iodide Live/Dead Assay

1. **Ki67 Cell Proliferation Assay (Immunocytochemistry)**
   1. Rinse the cell cultures with 0.1 M phosphate (PO_4) buffer for one minute two times. Fix the culture with 4% paraformaldehyde (PFA) for 20 min at room temperature. Remove the PFA and rinse the wells with PBS for seven minutes three times.
   2. After the final rinse, add 100 µl of blocker solution (phosphate buffer saline, 5% normal donkey serum, 0.4% bovine albumin serum, and 0.2% Triton X-100) to each well and incubate at room temperature for 1 hr. Prepare the primary antibody, rabbit anti-Ki67, by diluting in blocker solution at a working ratio of 1:200.
   3. Remove the blocker solution and apply 100 µl of the primary antibody solution to each well. Cover the 96-well plate and incubate the samples at 4 °C overnight.
   4. On the following day, remove the antibody solution and rinse with PBS for 7 min, 3 times.
   5. Prepare the secondary antibody, donkey anti-rabbit Cy3 in blocking solution at a working ratio of 1:500. Add DAPI nuclear stain to the secondary antibody solution at a dilution of 1:100. After the removal of the last PBS rinse, apply 100 µl of the secondary antibody/DAPI solution to each well. Incubate at room temperature in the dark for 90 min.
   6. Remove the secondary antibody/DAPI solution and rinse each well with PBS for 7 min, 3 times. Cover the 96-well plate and store at 4 °C until imaging.

2. **Propidium Iodide Live/Dead Assay**
   1. Measure cell death by propidium iodide (PI) exclusion assay as described below.
   2. Prepare the propidium iodide stain solution at a concentration of 1.5 µM in culture media.
   3. Add 100 µl of 70% ethanol to one well of the MSCs for 2 min with the intention of killing those cells. This well serves as a positive control for the PI stain. The majority of the MSCs will be PI-stained indicating cell death. Remove the ethanol solution.
   4. Add 100 µl of propidium iodide stain solution to each well and incubate for 20 min at 37 °C in a 5% CO_2 incubator.
   5. Rinse the cells with 0.1 M phosphate buffer for 1 min, 2 times. Fix the cells with 4% PFA in 0.1 M PO_4 buffer for 20 min at room temperature. Remove the PFA and rinse with PBS three times for 7 min.
   6. Incubate with DAPI solution (1:50) diluted in blocker solution for 1 hr at room temperature. Rinse all wells with PBS for 7 min, 3 times.
   7. Remove the DAPI solution and rinse each well with PBS for 7 min, 3 times. Cover the 96-well plate and store at 4 °C until imaging.

4. Automated Imaging and Multiwavelength Scoring Analysis

1. Load a 96-well plate (previously processed for Ki67 immunolabeling or propidium iodide-stained) into the HCS system and allow the plate to equilibrate for 20 min Open the HCS system image acquisition and analysis software.
2. Choose the acquisition settings for the 10X objective using camera binning at 1 and a gain setting of 2. Find the Z-plane in which the cells reside by utilizing the Auto Exposure function and calculate the offset for each wavelength of interest. For this analysis, capture images for DAPI (W1), Cy3 (W2), and FITC (W3). Choose the maximum intensity level at which the negative control wells show no signal for image acquisition. Confirm this setting is appropriate for the positive wells.
3. Acquire plate. Capture images and store them in the image acquisition and analysis software's database.
4. Once images have been acquired, open image acquisition and analysis offline software and review plate data from the images acquired above.
5. Select the Multi-wavelength scoring analysis. Configure the minimum and maximum intensities for each wavelength.
   NOTE: DAPI detection should mark the staining around each visible nucleus. Cy3 should detect the positive cells with Ki67 immunoreactivity (IR) and should not detect IR under the negative controls. For Cy3 Ki67 IR, the approximate minimum width was 7 µm, approximate maximum width was 30 µm, the intensity above the local background was 150 gray levels, and the minimum stained area was 50 µm^2.
6. Run analysis for all positions. Export the data to view in spreadsheet.

5. Cell Tracking

1. Open image acquisition and analysis program and click on "Review Plate Data [DB]…", selecting the plate of interest.
2. View the data as "Time *vs.* Well".
3. Choose one of the sites from the "Sites" section by left clicking on the desired selection. Select "Transmitted…" under the "Wavelengths:". Right click on the target well in the 96-well plate template and click on "Load Images".
4. Track the cells by clicking on the "Apps" and then "Track Objects".
5. Use "Dynamic Data Exchange (DDE)" to choose the format to export the data, *e.g.,* Microsoft Excel.

6. Choose the tracking data to export, *e.g.,* elapsed time, object number, distance, time interval, velocity, absolute angle, distance to origin, delta x and delta y.
7. Tag each cell of interest with "Ctrl key + left click" on target cells.
8. Track cells. If necessary, stop/change improper tracking of cells with the "Esc" key and then adjust the settings.
9. After cell tracking is complete, save data with "Log Data".

Representative Results

MSC growth parameters were examined by culturing the different populations of MSCs on different substrates. The five different populations of MSC subtypes (MSCs, GFP-MSCs, BDNF-GFP-MSCs, GDNF-GFP-MSCs, and BDNF/GDNF-GFP-MSCs) were plated into 96-well tissue culture plates pre-coated with the different substrates as illustrated in **Figure 1**. After four days of culturing, the plates were fixed and immunolabeled and/or stained with the appropriate reagents and then examined using the HCS system and analyses conducted with the image acquisition and analysis software program.
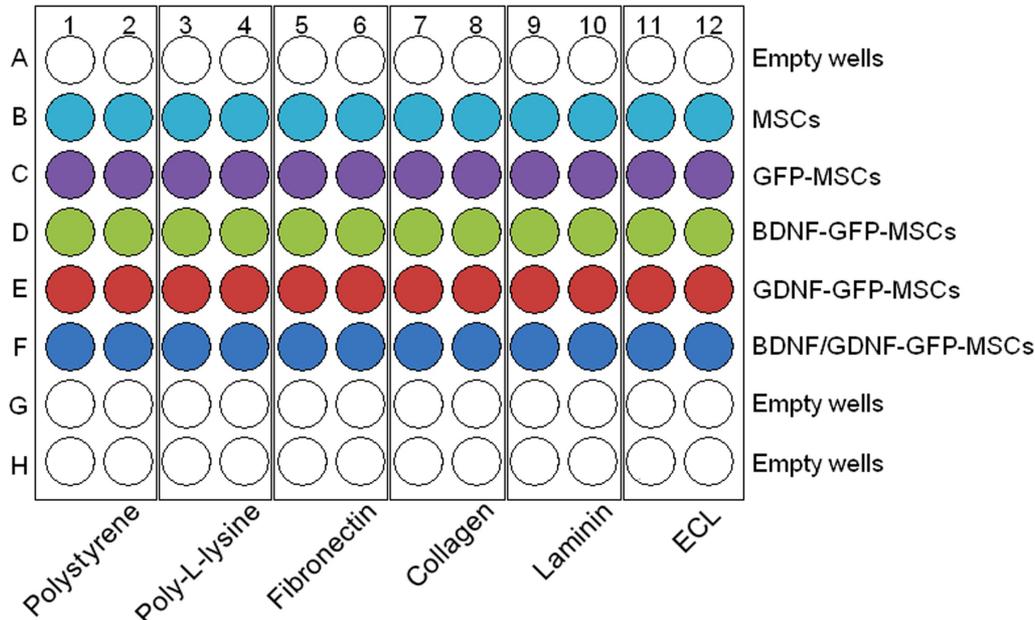

Figure 1: 96-well plate template for experimental design. 96-well plates were coated with various substrates and wells were seeded with engineered stem cells as shown in the template. As an example, only wells in rows B-F were used in this experiment. Rows A, G and H were left empty. Abbreviations – MSCs: mesenchymal stem cells; GFP-MSCs: green fluorescent protein-expressing MSCs; BDNF-GFP-MSCs: brain derived neurotrophic factor-GFP-expressing MSCs; GDNF-GFP-MSCs; Glial cell-derived neurotrophic factor-GFP-expressing MSCs; BDNF/GDNF-GFP-MSCs; BDNF and GDNF- GFP-expressing MSCs; ECL: Entactin-Collagen IV-Laminin).

Anti-Ki67 immunolabeling, followed by DAPI counterstaining, was used to evaluate whether the different substrates influenced proliferation of the different populations of engineered MSCs (**Figure 2A**). Expression of the Ki67 antigen occurs preferentially during late G_1, S, G_2 and M phases of the cell cycle, and is not detected in cells in the resting phase (G_0), and therefore is useful as a cellular marker for proliferation[15]. 4',6-diamidino-2-phenylindole (DAPI) is a commonly used nuclear and chromosome counterstain that emits blue fluorescence upon binding to AT regions of DNA[16]. The total number of cells in a field can be determined by counting the number of DAPI stained nuclei. As illustrated in **Figure 2B**, although there was variation in the percentages of proliferating MSCs, all substrates nevertheless supported considerable cell proliferation for each of the MSC subtypes.
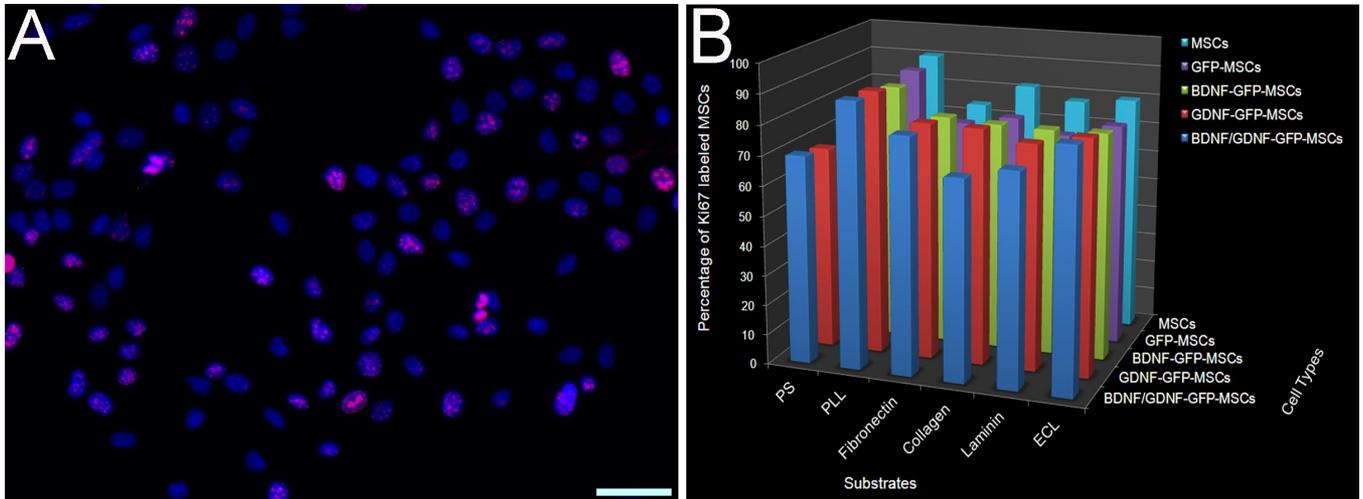
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Figure 2: Ki67 cell proliferation assay. (A) Merged, double-fluorescent image of Ki67 immunolabeling (red) and DAPI (blue) nuclear staining. Many of the MSCs were immunolabeled with the Ki67 antibody (red). Scale bar = 50 μm. **(B)** Bar graph illustrating the percentages of Ki67 immunolabeled MSC subtypes grown on polystyrene (PS), poly-L-lysine (PLL), fibronectin, collagen type I, laminin, or entactin-collagen IV-laminin (ECL) substrates for 5 days *in vitro* (DIV). N = one experiment. Each bar represents averaged pooled data from 8 imaged sites from 2 wells for each condition. Please click here to view a larger version of this figure.

Propidium iodide (PI) staining was used to evaluate whether different substrates influenced cell survival (**Figure 3**). Propidium iodide is a commonly used red-fluorescent nuclear and chromosome counterstain. Propidium iodide is membrane impermeant and generally excluded from viable cells, and thus is useful to detect dead cells in a population. The proportion of dead cells within a given condition can be determined when combined with a general nuclear label such as DAPI to identify all cells within a field. The percentage of PI-positive cells was low on all substrates examined (**Figure 3**). As a positive control for the PI reagent, a few wells containing MSCs were incubated in 70% ethanol, a condition known to kill most cells, resulting in a high percentage of PI-labeled cells as illustrated in **Figure 3B and 3C** (ethanol treated positive control).
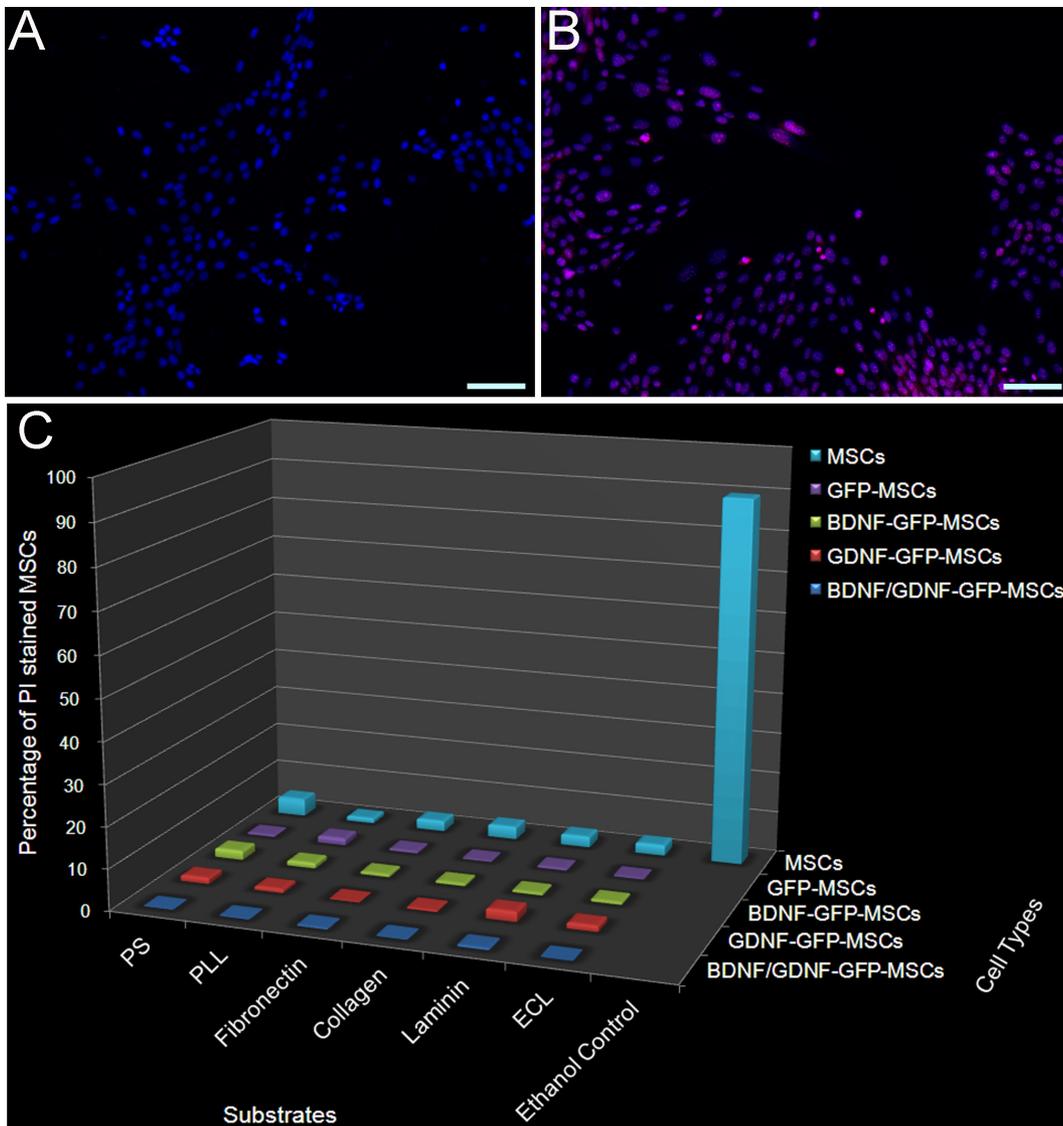
Figure 3: Propidium iodide cell death assay. (A) Merged, double fluorescent image for propidium iodide (red) and DAPI (blue) staining. Although the nuclei of all of the viable cells were stained with DAPI (blue), no propidium iodide staining was detected in the MSCs. **(B)** Virtually all MSCs were stained with propidium iodide following exposure to 70% ethanol. Scale bars in A and B = 100 μm. **(C)** Bar graph illustrating the percentages of propidium iodide (PI) stained MSC subtypes grown on polystyrene (PS), poly-L-lysine (PLL), fibronectin, collagen type I, laminin, or entactin-collagen IV-laminin (ECL) substrates for 5 days *in vitro* (DIV). Ethanol Control: This condition served as a positive control for the PI staining reagent. Most cells subjected to PI stain following ethanol treatment are dead and thus positively stained for the PI reagent. N = one experiment. Each bar represents averaged pooled data from 8 imaged sites from 2 wells for each condition. Please click here to view a larger version of this figure.

To investigate the possible influence of different substrates on the behavior of engineered MSCs, cell migration was analyzed using time-lapse digital microscopy and the transmitted light/environmental chamber system on the HCS system (see **Supplemental Video 1**). Multiple sites/well were time-lapse imaged and used to calculate cell migration rates for the different subpopulations of MSCs growing on the different substrates using the image acquisition and analysis software program. In general, as shown in **Figure 4C**, all subtypes of MSCs showed the fastest migration rate on the extracellular matrix-coated surfaces (Fibronectin, Collagen, Laminin and ECL) and the slowest on non-coated polystyrene surfaces.
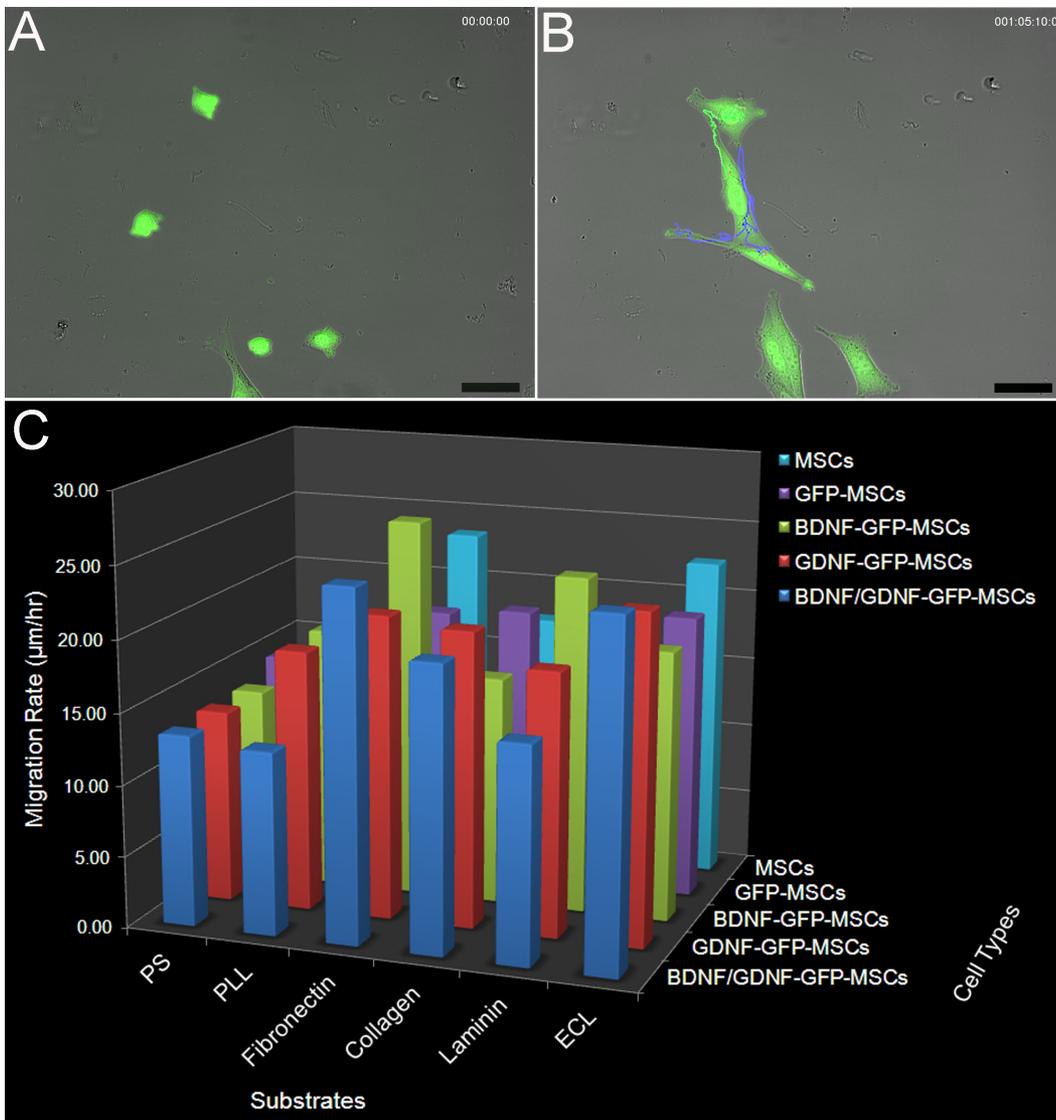
Figure 4: Cell tracking and migration. MSCs tracked with image acquisition and analysis software. Overlayed images of transmitted light and fluorescence images from **(A)** the start of time-lapse imaging and **(B)** at 29 hr later at the end of the time-lapse imaging session (see **Supplemental Video 1**). Cell migration tracks are indicated by the colored lines. Scale bar: 50 µm. **(C)** Bar graph illustrating the average migration rates (expressed as µm/hr) for MSC subtypes grown on polystyrene (PS), poly-L-lysine (PLL), fibronectin, collagen type I, laminin, or entactin-collagen IV-laminin (ECL) substrates for 2 days *in vitro* (DIV). N = one experiment. Each bar represents the average of at least 10 imaged cells from 2 wells for each condition. Please click here to view a larger version of this figure.

Taken together, these results provide preliminary evidence that these subpopulations of genetically engineered MSCs display similar growth properties. These results provide compelling evidence that the lentiviral mediated genetic modifications of these MSCs induced no dramatic detectable deleterious effects on the growth parameters investigated using this screening platform.

Supplemental Video 1. Time-lapse digital video of MSC tracking using the image acquisition and analysis software program. The migration path's for two MSCs [indicated as 1 (green tracking line) and 2 (Blue tracking line)] are illustrated. Video captured during a 29 hr period. Images were captured every 5 min. Fluorescence images of GFP-expressing MSCs was used for time-lapse imaging in preparing the video. Calibration bar = 50 µm. This type of analysis is useful for investigating cell behaviors, including cell migration and cell division.

Discussion

Adult mesenchymal stem cells (MSCs) are an attractive cell type for development of an experimental strategy combining a cellular and gene delivery based therapy. MSCs are multipotent, capable of differentiating into cells of mesodermal lineage, and display considerable plasticity, differentiating/transdifferentiating into neuronal and glial lineages with the appropriate induction paradigms[17,18]. Furthermore, MSCs have been transplanted and proven effective in preclinical studies for a number of disorders, including neurodegenerative conditions[19]. The therapeutic efficacy of MSCs is well known due to their beneficial anti-proliferative, anti-inflammatory and anti-apoptotic activities[20]. MSCs are also known to produce and secrete various neurotrophic and growth factors, which likely contributes to the neuroprotective qualities associated with naïve MSCs following transplantation at sites of injury or disease[21]. Importantly, MSCs can be genetically modified for sustained delivery of

neurotrophic factors for combined cellular and gene therapy-based applications and have been used in a number of animal models of injury or disease to the CNS[11,19,22].

In developing a combined cellular and gene therapy-based strategy, it is important that the health of the cells is carefully assessed prior to their extensive use for *in vitro,* and especially *in vivo* applications. As a proof of concept, we investigated multiple populations of engineered and control MSC lines in order to study the consequences of the genetic modifications on cell health and fitness using a high content screening (HCS) approach. In general, HCS refers to cellular image-based high throughput screening[14]. This screening approach permits a quantitative assessment of cellular phenotypes at multiple levels of spatial (cell to subcellular) and temporal (milliseconds to days) resolution across various experimental conditions. Using this approach we accessed possible differences in substrate preference on the following parameters: cell proliferation, expression of green fluorescent protein (GFP), cell death, and cell motility/migration. Experiments were designed in 96-well cell culture plate format. Within a single plate we routinely investigated possible substrate-related differences with respect to each parameter for the different MSC populations of lentiviral-transduced cells and compared the engineered MSCs with the original, non-transduced MSCs. This provided a means to directly compare the results for the different MSC subtypes with a battery of *in vitro* assays such as cell proliferation using Ki67 immunolabeling, live/dead cell viability assay using propidium iodide staining, and cell behavior by performing time-lapse digital imaging. As an extension of this HCS one can also perform ELISAs on conditioned media samples collected from individual wells to quantitatively determine secretion of neurotrophic factors. Conditioned media from different MSC subtypes may also be used in *in vitro* bioassays to determine biological activity of secreted factors[11,23]. This type of HCS platform may also be used for *in vitro* measurements of neurite outgrowth from primary neuronal cultures and neural stem cell lines[24]. Overall, our results demonstrated that the subpopulations of the genetically modified MSCs displayed similar behaviors in comparison to the non-modified MSCs. The influence of diverse culture substrates on cell growth and cell migration was not dramatically different between the MSC subtypes, as well as culture substrates. As such, the extracellular matrix substrates tested did not appear to play a critical role in modulating these aspects of cell behavior for these different engineered MSCs.

This study demonstrates the use of an HCS system for analyzing different aspects of cell behavior. However, it is not uncommon to encounter limitations associated with image analysis. On occasion, while analyzing the fluorescence images, it was somewhat difficult to determine the correct threshold value above which immunolabeled or stained cells would be counted as positively labeled/stained. Thus, to minimize subjective bias, the determination of threshold values was dependent upon a comparison with controls (negative controls for fluorescence imaging were carried out in parallel during all processing by the omission of the primary or secondary antibodies). Another limitation was encountered during the analysis of cell migration using time-lapse digital imaging. In some cases, the imaging software was not able to differentiate between random Brownian motion of a cell versus a cell actually migrating only a very short distance. Additional limitations were evident in situations where the analysis software was not able to distinguish the presence of multiple cells in very close proximity to one-another. To overcome this limitation required manual cell selection during the analysis rather than a fully automated analysis. Cell plating density can also result in skewing cell migration data between populations of cells that display greater preference to grow in clumps versus cells that grow in isolation from each other. These types of differences are in part likely a reflection of cell-substrate versus cell-cell preferences.

Using an HCS system to acquire images and perform data analysis provides an efficient and rapid means to assess multiple cell parameters. In addition, time-lapse digital videos for 30 different conditions (6 substrates and 5 different MSC subtypes) were routinely acquired for periods ranging from hours to days (48 hr) while using the environmental chamber. This data was subsequently used to calculate and determine differences in cell migration rates across various cell lines on different ECM molecules.

In this report we have highlighted the implementation of a high content screening platform to assess cell health and function. This type of analysis is useful for developing rational strategies for designing cell-types, as well as polymer substrates to facilitate directed cell growth and neural regeneration. This is an essential step towards application of stem cell-based delivery of therapeutic factors prior to extensive *in vivo* preclinical studies using cell transplantation strategies.

Disclosures

Acknowledgements

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