Adhesive Signature Technology for iPSC-Derived Neuronal Cells in Regenerative Medicine

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Statement of Purpose: Human stem cells are an invaluable tool for biomedical research because of their role as (i) cell sources for countless therapeutic applications, (ii) models for studying human development and disease, (iii) and targets for drug testing and development. This is especially true for neural cells. A promising source for these cells is human-induced pluripotent stem cells (hiPSC), which can be differentiated into neural rosettes. However, a major bottleneck for neural stem cell research is the lack of a rapid, label-free, and efficient method for purifying neural cells derived *in vitro* from hiPSCs. The objective of this project is to develop an effective and scalable technology to purify hiPSC-derived neuronal cells at various stages of differentiation for use in either therapeutic procedures or as disease modeling tools. We hypothesize that neural cells at specific differentiation stages will have distinct adhesive force signatures that can be exploited by the µSHEAR (micro–stem cell high-efficiency adhesion-based recovery) system to selectively purify them with high purity and yield. The innovation of this project lies in the integration of unique bioengineering methodologies and stem cell biology to generate a robust purification platform for neural stem cells.

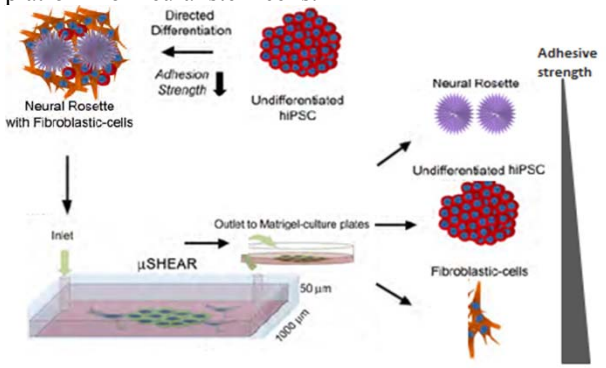

Fig. 1 *µSHEAR system for neural purification*

Methods: Neural rosettes were derived from feeder-free, hiPSCs [1]. The microfluidic channels were sterilized and coated with laminin [1]. The hiPSC derived neural culture was mechanically disassociated into smaller colonies, pipetted into the inlet reservoir using a 200 µL pipette tip and cultured in the device for 24h before detachment experiments. Cells were exposed to fluid flow at predetermined PBS flow rates [1]. Recovered cell/colonies were plated on Matrigel-coated plates containing neural differentiation media. After 24 hours, the cells where stained for Pax6, Oct4, Sox1, and DAPI. To determine population composition, collected cells were immediately disassociated with 0.25% trypsin, stained against Pax6 and Sox1, and analyzed using flow cytometry

Results: The µSHEAR microfluidic system allows for the rapid and high yield purification of different cell types based on their unique "adhesive force signatures" [1]. The hiPSC-derived neural rosettes were introduced into the µSHEAR devices and survived within the devices for up to 48hr. This process did no disrupt the rosette structure (Fig. 2A-D). Recovered neural rosettes stained positive for neural markers Pax6 and Sox1, but negative for pluripotent marker Oct4 (Fig. 2E). Even after purification the rosette structure remained intact. The initial hiPSC differentiated population containing the neural rosettes was analyzed by flow cytometry (Fig. 2F). Around 10% of the population expressed neural rosette markers, while there were significant subpopulations of Pax+/Sox- and Pax-/Sox- cells.

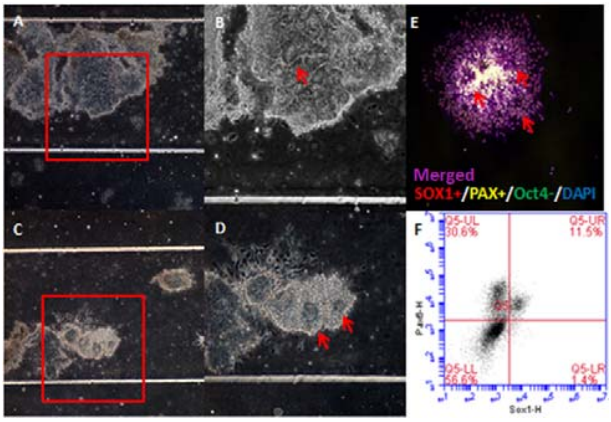

Fig. 2 *Neural rosettes are successfully introduced into the µSHEAR microfluidic devices and maintained after recovery. A-D) Neuronal cells are shown 24hr post introduction into µSHEAR microfluidic devices. The red arrows indicate intact rosette structures. E) Merged image of rosette structure 24hr post recovery from µSHEAR devices stained for nuclear stain DAPI (blue), hiPSC marker OCT4 (green) and neural markers SOX1 (red) and PAX6 (yellow). F) Pax6 (y-axis) and Sox1 (x-axis) flow cytometry staining of initial rosette population*

Conclusions: We have demonstrated the feasibility of applying the µSHEAR system for the purification of neural cells. When hiPSC-derived neural cells were introduced into the µSHEAR devices they (i) remained alive, (ii) stained positive for the relevant markers, and (iii) retained the rosette structure both within the devices and after recovery. Furthermore, the composition of the original population was characterized and contained a 10% population positive for rosette markers. Purification studies are ongoing. Future studies are aimed at characterizing the differentiation potential of the purified rosettes and benchmarking the µSHEAR technology to other purification techniques.

References:

1. Singh A *et al*, *Nature Methods* 2013, 10(5):438-44

Funding Sources

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