Differential adhesion strength as a molecular signature for rapid separation of partially reprogrammed cells from human stem cell reprogramming cultures

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Statement of Purpose: Cellular reprogramming of somatic cells such has human fibroblasts to hiPSCs using reprogramming factors (e.g., *OCT3/4*, *SOX2, KLF4,* and *c-MYC*) result in highly heterogeneous cultures consisting of fully reprogrammed cells along with partially reprogrammed and non-reprogrammed cells. Despite numerous advances, the reprogramming process remains inefficient (0.001-2%). hiPSC survival and stemness require compact colonies with cell-cell adhesion, therefore single cell sorting methods like FACS are not suitable. Furthermore, enzymatic methods are non-selective, causes karyotypic abnormalities compared to manual passaging and require re-aggregation of hPSCs for improved survival. A recent paradigm shift in stem cell field has been the findings that pluripotent stem cells can differentiate into terminal tissue cells when exposed to intrinsic properties of the ECM and mechanical forces. However, less is known about how cell mechanics changes with reprogramming. We hypothesized that that alterations in the cell adhesivity to a substrate occur coincidentally with hiPSC reprogramming and is a function of pluripotent state of the reprogrammed cell. We demonstrated a unique "adhesive signature" for cells undergoing reprogramming and differentiation that is a multifactorial function of ECM-bound integrins, assembly of focal adhesions, and the resulting cell-ECM adhesion strength [1]. We engineered a platform microfluidic technology that exploited the differences in the adhesion strength among non-reprogrammed, partially reprogrammed cells and bona fide hiPSCs to selectively isolate pure hiPSCs [1].

Methods: Reprogramming culture was pipetted into the inlet reservoir and cultured in the device for 24h before detachment experiments. Cells were exposed to fluid flow at predetermined PBS flow rates [1].

Results: During reprogramming of human lung fibroblasts (IMR90) to pluripotency we observed < 1% conversion to fully reprogrammed hiPSCs positive for cellular markers that define complete reprogramming [1]. Fibroblasts possessed actin stress fibers and vinculin enriched at FA. In contrast, hiPSCs exhibited significantly fewer actin fibers with diffused vinculin throughout the cytoplasm. Non-pluripotent cells in reprogramming cultures exhibited mixed regions of well-defined FA in spread cells and round cells without distinct focal adhesions. Analyses among fibroblastic cells and hiPSCs revealed significantly lower adhesion strength to fibronectin, laminin, and Matrigel substrates for hiPSCs compared to fibroblasts ($P < 0.02$), indicating that cells shift in their adhesive properties during reprogramming. We exploited the unique adhesive signatures of hiPSCs to isolate fully reprogrammed undifferentiated hPSC colonies from a heterogeneous cell population. We employed adhesive force-based separation via a simple microfluidic system termed µSHEAR (micro Stem cell High-Efficiency Adhesion-based Recovery), a label-free technique that requires minimal cell processing (**Fig. 1A**). Application of laminar flow using PBS generated fluid shear stresses on adherent cells within the device. Fully reprogrammed hiPSC colonies detached at a shear stress of 85–125 dynes cm^{-2} within 4–5 min of applying fluid flow **(Fig. 1B)** whereas partially reprogrammed cells remained attached. Flow cytometry analysis of isolated hiPSCs indicated 95-99% purity and isolated cells expressed TRA-1-60, TRA-1-81, DNMT3B, REX1, OCT4, SSEA4, GDF3, hTERT, and NANOG, indicating that they were fully reprogrammed. Residual cells within the device expressed OCT4, hTERT, and GDF3 but not the other markers (**Fig 1C**). µSHEAR-isolated hiPSCs displayed unmethylated OCT4, SOX2, NANOG, retained pluripotency and self-renewal characteristics, and formed teratomas when implanted into immunodeficient mice (**Fig. 1D**).

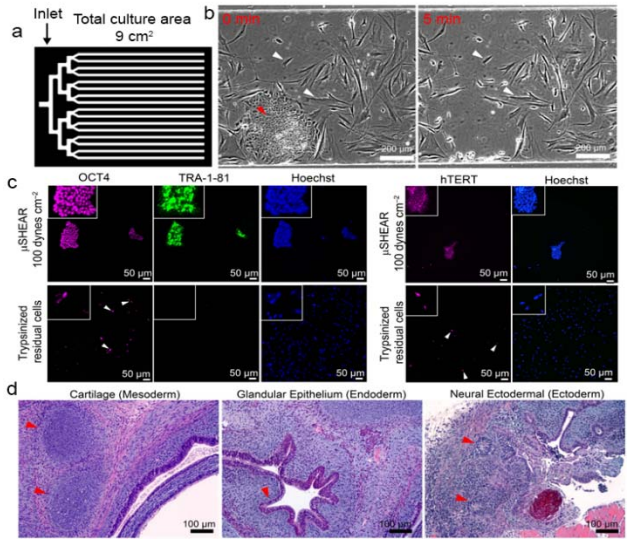

Fig 1. (A) Schematic of µSHEAR device (B) Heterogeneous reprogramming culture seeded into a µSHEAR device and subjected to a shear stress of 100 dynes cm^{-2} for 5 min. Red arrowhead:hiPSC colony. White arrowheads: partially reprogrammed cells. (C) µSHEAR-separated partially reprogrammed cells expressed Oct4 and hTERT but were negative for TRA-1-81. (D) Representative H/E–stained sections from a teratoma produced from µSHEAR-isolated hiPSCs indicating all three germ layers.[1]

Conclusions: These studies demonstrate that fully reprogrammed hiPSCs can be selectively isolated from residual nonreprogrammed and partially reprogrammed cells using µSHEAR on the basis of differences in adhesion strength.

References:

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

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