FROM MANUAL TO AUTOMATED: REVOLUTIONIZING HIPSC DIFFERENTIATION BY INCREASING EFFICIENCY AND FIDELITY FOR NPCS, HSPCS, AND LPCS, WITH CELLRAFT TECHNOLOGY

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- **Automate hiPSC workflows for multi-lineage differentiations**
- **Live stain for selection and confirmation**
- **Select for monoclonality, morphology, colony size, and phenotype with analytical software**
- **Reduce population heterogeneity and increase total yield**

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BACKGROUND

Differentiation of patient-derived and genetically modified hiPSCs provides unmatched potential in developing accurate disease models. Despite numerous available protocols and commercial kits for hiPSC differentiation, the process of differentiating hiPSCs is highly technical, often requiring manual manipulation, which can lead to inefficiency and lack of reproducibility. We hypothesized that CellRaft® technology could overcome technical challenges in differentiating hiPSCs into cell types representing each lineage. Using the CellRaft Array and the CellRaft AIR® System, protocols were developed to improve efficiency, providing an automated solution for these manual workflows. To optimize neural progenitor (NPC) differentiation, embryoid bodies were seeded on CellRaft Arrays in neural induction media to monitor formation of neural rosettes. Automated isolation of CellRafts containing neural rosettes was performed using the AIR System, eliminating the need for manual dissection or rosette selection reagents. Post-isolation staining revealed 100% of CellRafts containing neural rosettes were Pax6 positive, indicating high fidelity of NPC differentiation. For efficient hematopoietic progenitor (HSPC) differentiation, manual selection of 100-200µm aggregates is a critical step requiring precise timing and collection methods. We utilized software tools selecting size-specific monoclonal hiPSC colonies for isolation into collections plates for HSPC differentiation. This automated method increased colony survival by 100% compared to the standard protocol with >100,000 monoclonal CD34+ HSPCs per selected colony within 2 weeks. During lung progenitor (LPC) differentiation, a high degree of heterogeneity exists within the population in the mesendoderm state necessitating purification after differentiation. Using the CellRaft Array, we stained for the endoderm-specific marker, CXCR4 and selected populations for isolation using the AIR System, increasing the accuracy of LPC differentiation. These data demonstrate how automating the manual steps of hiPSC differentiation, including image-based monitoring, phenotypic selection, and isolation, increases the efficiency and fidelity of hiPSC differentiation, which accelerates the development of clinically relevant models.

METHODS

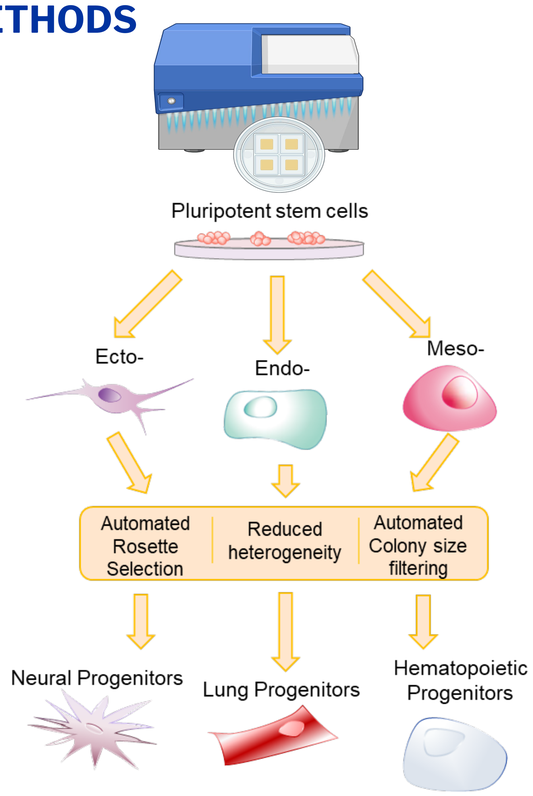
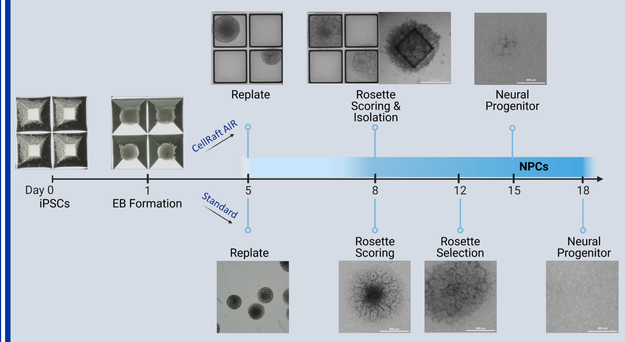


Figure 1: Graphical Abstract of Differentiation on the CellRaft Array. iPSCs were differentiated using STEMDiff NPC, HSPC, and LPC Differentiation Kits commercially available from StemCell Technologies. Workflows typically require manual steps to obtain desired differentiated cell types and were all automated using CellRaft Technology.

Ectoderm



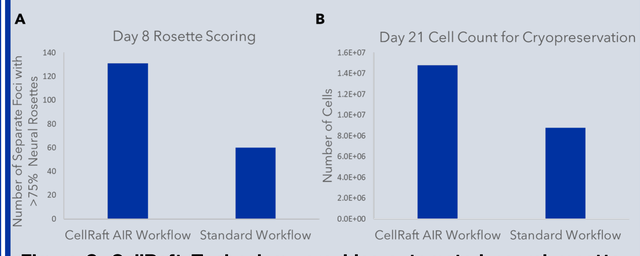


Figure 2. CellRaft Technology provides automated neural rosette selection and increases neural progenitor yield compared to the standard workflow. KYOU-DXR01089B (ACS-1023) hiPSC EBs were either seeded onto the CellRaft Array or plated according to the standard NIM + SMADi workflow. On day 8 (A) the CellRaft Array contained more than double the number of neural rosettes with >75% neural rosette morphology, compared to the companion 6-well plate. Additionally, the CellRaft workflow resulted in >1.6-fold increase in total number of cells available for cryopreservation on Day 21 after 2 passages in Neural Progenitor Media (B).

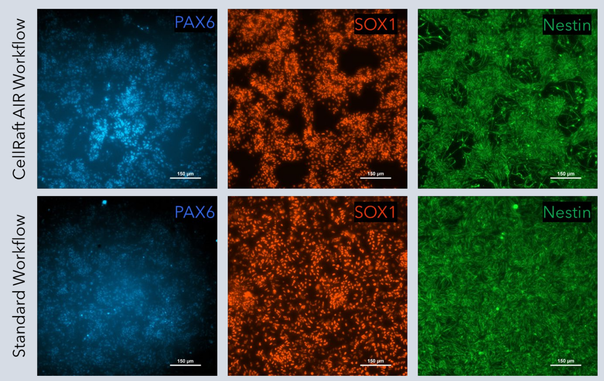


Figure 3: Differentiated neural progenitor cells demonstrate canonical neural marker expression. NPCs (p2, Day 24) generated using the CellRaft AIR and standard workflows were stained for neural progenitor markers PAX6, SOX1, and Nestin to validate neural induction.

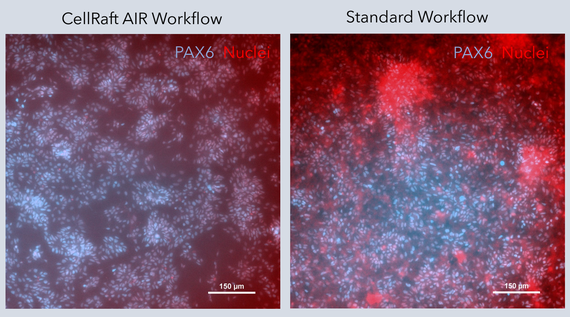


Figure 4: The CellRaft AIR workflow generates a homogeneous neural progenitor cell population compared to the standard workflow. NPCs (p2, Day 24) generated using the CellRaft AIR and standard workflows were stained for neural progenitor marker PAX6 and counterstained with a NucRed (647) nuclear dye. The CellRaft AIR population exhibits canonical rosette morphology, as well as more consistent overlay of PAX6 positive nuclei and total nuclei indicating a more homogeneous population of cells compared to the standard workflow.

Endoderm

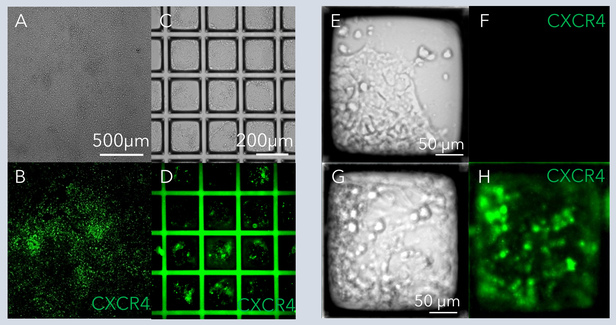


Figure 5: CXCR4 expression shows differentiation heterogeneity in definitive endoderm. Endoderm differentiation traditionally has heterogenous CXCR4 expression (A-B) 24-wp. Similar heterogeneity was observed on a 200 µm Quad Array (C-D). CXCR4 proved to be negative in undifferentiated iPSCs (KYOU-DXR01089B (ACS-1023)) (E-F) while differentiated cells expressed positive CXCR4 (G-H) indicating it could be used as a selection marker in LPC Differentiation.

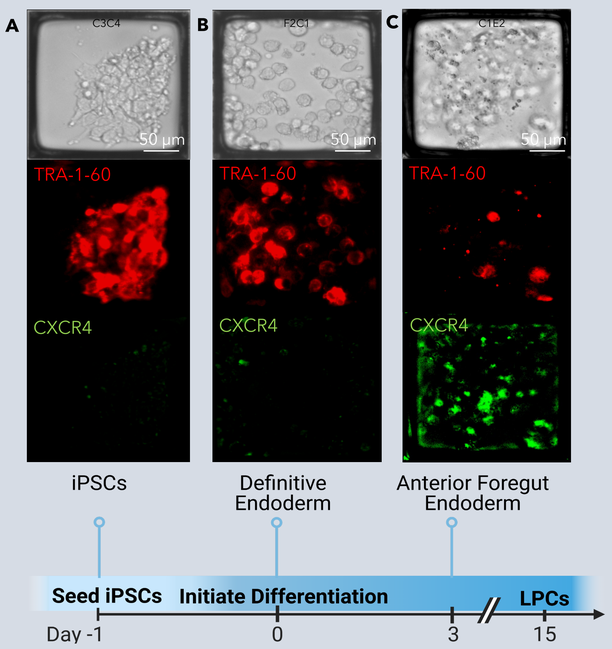


Figure 6: TRA-1-60 and CXCR4 expression is easily visualized throughout differentiation to lung progenitor cells. iPSCs were differentiated to LPCs and stained on D(-1), D0, and D3 to follow differentiation progress on a 200 µm CellRaft Array. Over time, TRA-1-60 expression decreased while CXCR4 expression increased (A-C) and was identified using CellRaft Cytometry Software.

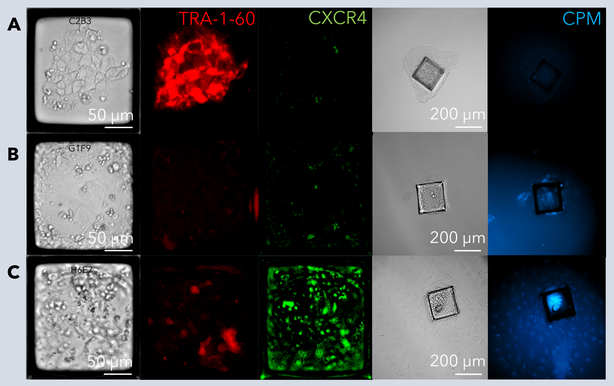


Figure 7: Selection of high CXCR4 expression on Day 3 benefits lung progenitor differentiation potential. Using the CellRaft Array, cells were stained for selection markers TRA-1-60 (red) and CXCR4 (green) on D3. Undifferentiated (A), low-CXCR4 (B), and high-CXCR4 (C) colonies were isolated and transferred to a 96-wp using the AIR System. Differentiation continued in the 96-wp where high-CXCR4 isolated colonies contained more established colonies that were positive for LPC differentiation marker CPM (blue) on D15.

Mesoderm

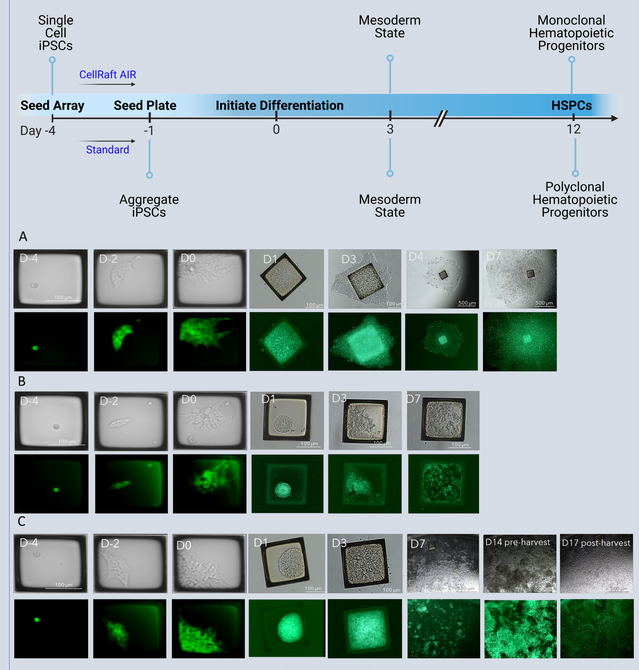


Figure 8: Automated colony size selection increases hematopoietic stem and progenitor cell differentiation, survival and efficiency. β-actin-GFP monoclonal hiPSC aggregates (IPSC1030) cultured on a CellRaft Array (D-4 – D0) then automatically selected and isolated by size using CellRaft Technology for HSPC differentiation (D1 – D17). Untreated monoclonal IPSC1030 in pluripotent conditions had healthy pluripotent colony growth (A). Selection of aggregates under 200 µm (B) for HSPC differentiation resulted in cell death and differentiation failure. Aggregates over 200µm resulted in successful HSPC differentiation (C) with one hiPSC generating over 100,000 HSPCs post-CD34+ magnetic selection by D14 (data not shown).

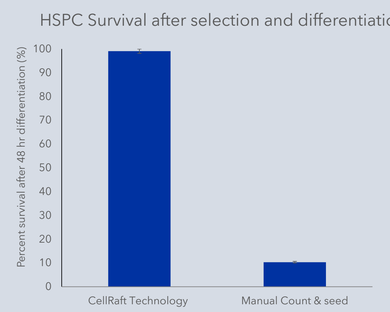


Figure 9: CellRaft Technology significantly increases survival of hiPSC aggregates undergoing HSPC differentiation stimuli compared to standard manual workflow. KYOU-DXR01089B (ACS-1023) hiPSC aggregates over 200µm were either manually counted and seeded using dissociation reagent or automatically selected and isolated using CellRaft Technology. HSPC differentiation stimuli was added for 48 hours, and surviving colonies were accessed.

CONCLUSIONS

- CellRaft Technology automates laborious manual differentiation workflows for efficient and simplified generation of hiPSC-derived cell types.
- The CellRaft AIR enables imaged-based monitoring of differentiation, software-guided phenotypic selection, and automated isolation with a single instrument.
- CellRaft Cytometry software provides quantitative analysis with customizable algorithms to detect and filter for monoclonality, aggregate size, fluorescence, and morphological features.
- The automated workflows for NPC, LPC, and HPSC differentiation increased cell yields, improved homogeneity of differentiation, and shortened experimental timelines compared to the traditional manual workflows.

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