Efficient Differentiation of Human Pluripotent Stem Cells to Hematopoietic Progenitor Cells in Serum-Free Culture Conditions

Melanie Kardel[1], Rebecca J. Noort[1], Jenny Chen[1], Erik B. Hadley[1], Bert Wognum[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Sharon A. Louis[1]

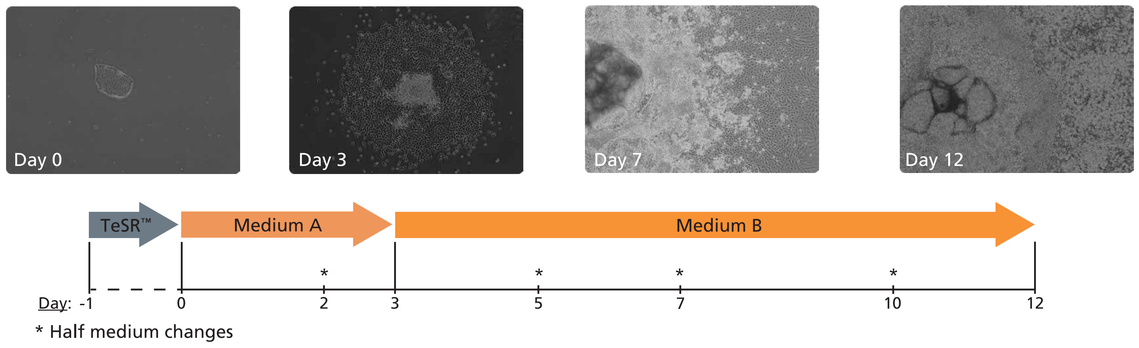
[1] STEMCELL Technologies Inc., Vancouver, BC, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Introduction

Reproducible protocols for differentiation of human pluripotent stem cells (hPSCs) to hematopoietic progenitor cells have been difficult to develop. Most protocols for hematopoietic differentiation use hPSCs maintained on feeder cells or rely on undefined serum-containing media or co-culture with stromal cells: all contributing factors to inconsistent results obtained across multiple hPSC lines. Here we demonstrate that hPSCs maintained using TeSR™ media can be efficiently differentiated to hematopoietic progenitor cells in defined, serum- and feeder-free conditions using the STEMdiff™ Hematopoietic Kit. Differentiation was efficient and reproducible across multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines.

Methods

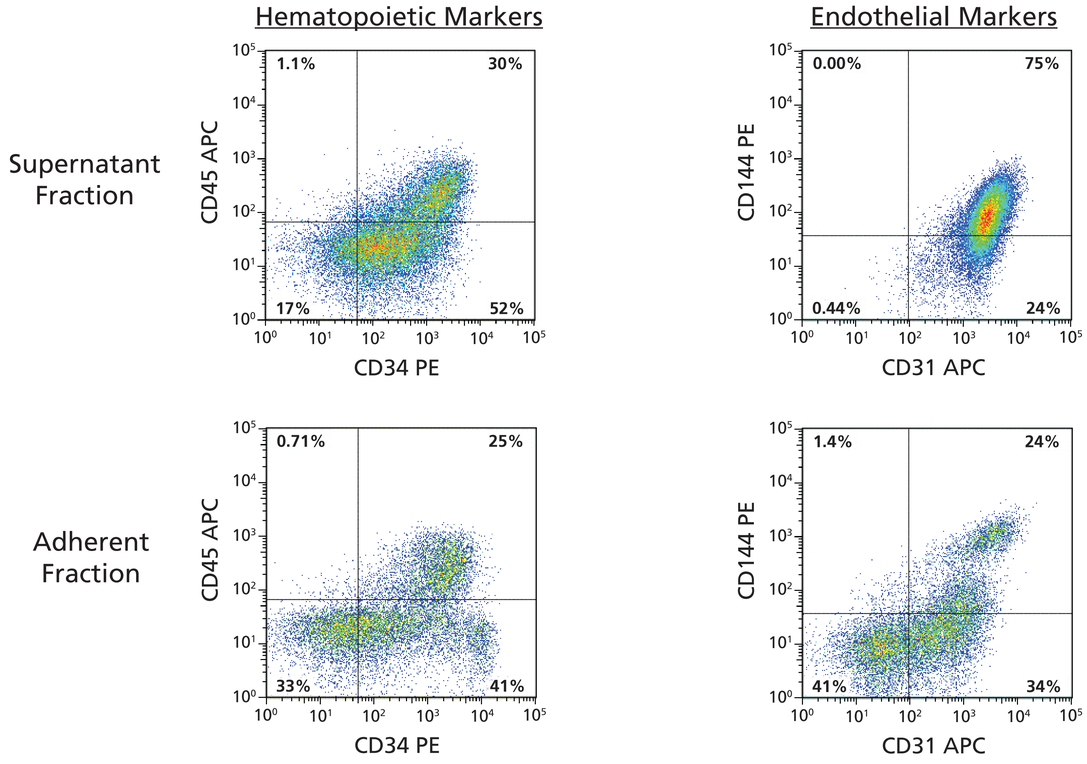
FIGURE 1: Protocol Schematic for STEMdiff™ Hematopoietic Kit



Human ES or iPS cells were plated as small aggregates (100 - 200 µm in diameter) at very low density (15 - 20 aggregates/cm^2) in mTeSR™1 or TeSR™-E8™ on Corning® Matrigel® and allowed to attach overnight. The next day, differentiation was initiated by changing the medium to STEMdiff™ Hematopoietic Medium A. After 3 days, medium was changed to STEMdiff™ Hematopoietic Medium B for the second stage of differentiation. Additional half medium exchanges were performed as indicated in the schematic (*). At day 12, differentiated cells were harvested from the supernatant and assessed by flow cytometry or in colony-forming unit (CFU) assays using MethoCult™ H4435 Enriched methylcellulose-based medium. In some experiments, the adherent layer was also harvested separately by dissociation with Accutase™ for similar characterization studies.

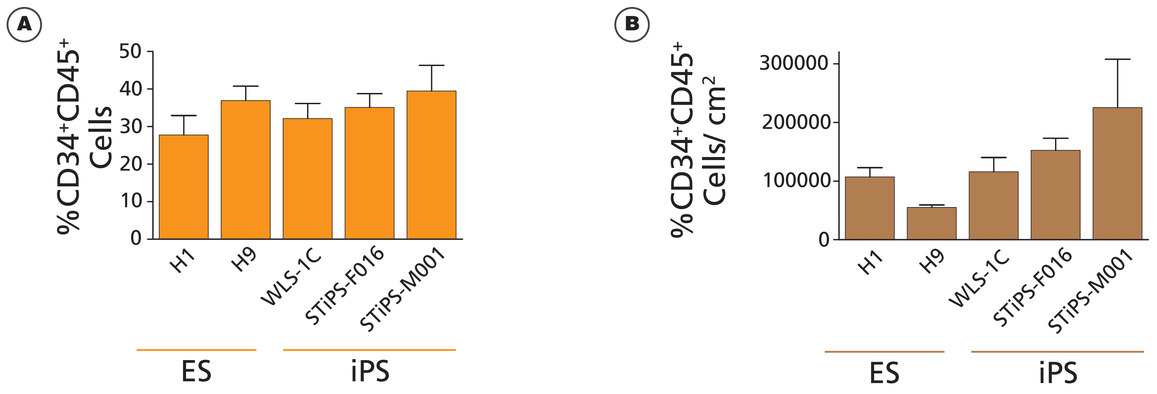
Results

FIGURE 2: Supernatant and Adherent Fractions Have Distinct Phenotypes and Contain Hematopoietic Cells



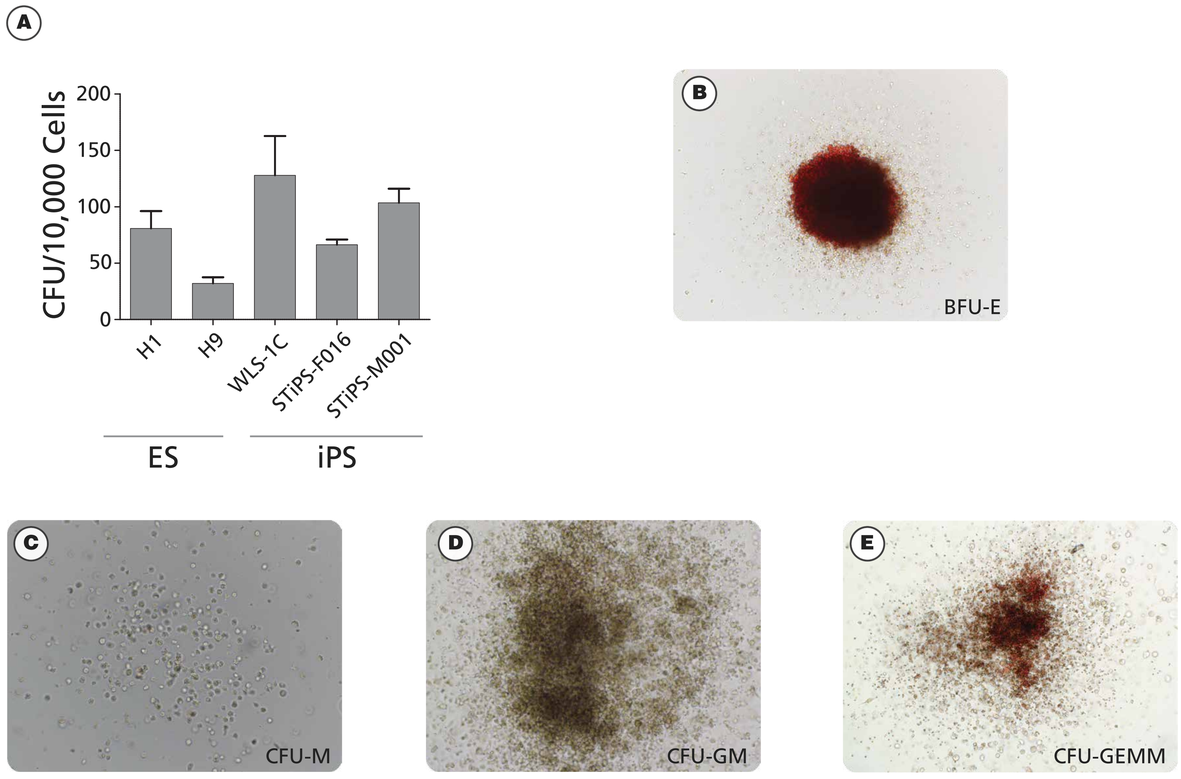
At day 12 of differentiation using the STEMdiff™ Hematopoietic Kit, cells were harvested separately from the supernatant and adherent fractions of the culture. Flow cytometry was used to assess the expression of hematopoietic markers CD34 and CD45, and endothelial cell markers CD144 (also known as VE-cadherin) and CD31 (also known as PECAM). In the adherent fraction (bottom row), CD34$^+$CD45$^+$ hematopoietic progenitors are detected, as well as a population of CD144$^+$CD31$^+$ endothelial cells. In the supernatant fraction (top row), where the majority of cells are recovered, a significant proportion of CD34$^+$CD45$^+$ cells is also observed. Residual low expression of CD144 likely indicates hematopoietic cells recently derived from hemogenic endothelium.

FIGURE 3: Efficient Generation of Hematopoietic Progenitors from Multiple Human ES and iPS Cell Lines



At day 12 of differentiation using the STEMdiff™ Hematopoietic Kit, cells were harvested from the supernatant and assessed for expression of hematopoietic markers CD34 and CD45 using flow cytometry. **A)** Summary of the average percentage CD34$^+$CD45$^+$ double-positive cells in the supernatant fraction. The overall average across cell lines was 36 ± 2%, n = 3 (H1), 8 (H9), 3 (WLS-1C), 4 (STiPS-F016), 6 (STiPS-M001). **B)** Summary of the yield of CD34$^+$CD45$^+$ hematopoietic progenitors in the supernatant fraction. The overall average across cell lines was 130,000 ± 24,000 cells/cm^2, equating to over 1 million hematopoietic progenitors per well of differentiation in a 6-well plate.

FIGURE 4: hPSC-Derived Hematopoietic Progenitor Cells Produce Colonies of Multiple Lineages



At day 12 of differentiation using the STEMdiff™ Hematopoietic Kit, cells were harvested from the supernatant and assessed in a CFU assay using MethoCult™ H4435 Enriched methylcellulose-based medium. **A)** Summary of the average CFU frequency in the supernatant fraction shown per 10,000 hPSC-derived hematopoietic cells harvested from the day 12 supernatant. The overall average total CFU frequency was 74 ± 9 CFU/10,000 cells, n = 3 (H1), 8 (H9), 3 (WLS-1C), 4 (STiPS-F016), 6 (STiPS-M001). Representative images of **B)** BFU-E, **C)** CFU-M, **D)** CFU-GM and **E)** CFU-GEMM colonies (all 40X magnification).

Summary

STEMdiff™ Hematopoietic Kit is a defined, serum-free and feeder-free media formulation for:

- Efficient generation of hematopoietic progenitors including CFUs in less than two weeks
- Robust generation of hematopoietic progenitors across multiple human ES and iPS cell lines
- A simple monolayer-based protocol producing easy to harvest hPSC-derived hematopoietic cells in suspension

TOLL-FREE PHONE 1 800 667 0322 • PHONE 1 604 877 0713 • INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE