STEMDIFF™ ENDOTHELIAL: A NEW CULTURE WORKFLOW FOR EFFICIENT DERIVATION AND EXPANSION OF ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Marchetti V.[1], Lee K.[1], Wagey R.[1], Szilvassy S.J.[1], Thomas T.E.[1], Eaves A.C.[1,2] and Louis S.A.[1]

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

Introduction

Endothelial Cells (ECs) are typically isolated from several tissues such as the heart, brain, lungs, liver, and kidney. However, their low frequency, heterogeneity, and the loss of phenotype and functionality after isolation have prompted researchers to use human pluripotent stem cells (hPSCs) as an alternative source. Current protocols for deriving endothelial cells from hPSCs involve culturing cells in serum-containing medium, purification of cells before and after culture, and prolonged culture times. Endothelial cells generated using such methods generally exhibit limited proliferation, lose their characteristic phenotype, and stop dividing early during passaging. Additionally, undesired mesenchymal cell types often arise in these cultures. We have developed a method to efficiently induce derivation of ECs from human induced pluripotent stem (iPS) cells and embryonic stem (ES) cells under defined xeno-free culture conditions.

Materials and Methods

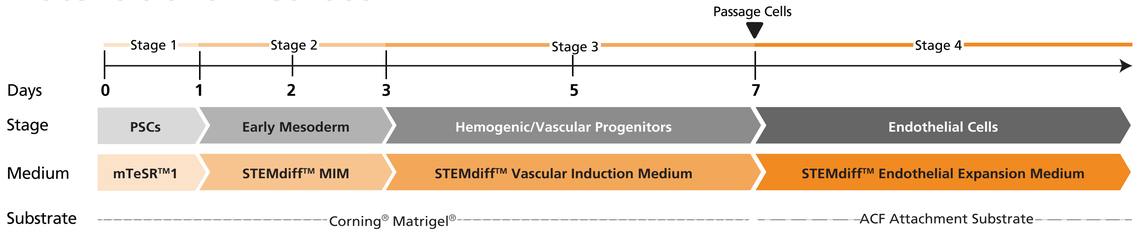

FIGURE 1. STEMdiff™ Endothelial Workflow

hPSCs are seeded as single cells at 7,500 to 12,500 cells/cm^2 in mTeSR™1 with Y-27632 (ROCK inhibitor) for 24 hours (Stage 1). Differentiation is then initiated by replacing medium with STEMdiff™ Mesoderm Induction Medium (MIM) for 2 days (Stage 2). On day 3, vascular induction is initiated by replacing medium with STEMdiff™ Vascular Induction Medium for 4 days (Stage 3). On day 7, cells are harvested and the expected percentage of CD34$^+$ cells is > 60%. The cells are expanded in STEMdiff™ Endothelial Expansion Medium or commercially available serum-containing medium without any selection or purification on ACF Attachment Substrate (Stage 4). The proliferative potential of hPSC-derived ECs in each medium was measured by counting cells at each passage for up to 6 - 7 passages. The growth curve was generated by multiplying the initial number of cells by fold expansion for each passage cumulatively. The doubling rate was calculated using the formula = N/(3.3*LOG(C2/C1)); N = number of days in culture; C1 = initial cell concentration; C2 = final cell concentration. Experiments using two iPS cell lines (1C and M001) and two ES cell lines (H9 and H7) demonstrated that the average fold expansion at each subculture (P1 - P8) was ~4.5. After the first passage, 99% of cells express CD34, CD31, and CD144 and they maintain this endothelial phenotype for 6 - 8 passages as analyzed by flow cytometry. The hPSC-derived endothelial cells are functionally active and able to incorporate acetylated LDL at early (P2) and late (P6) passage.

Results

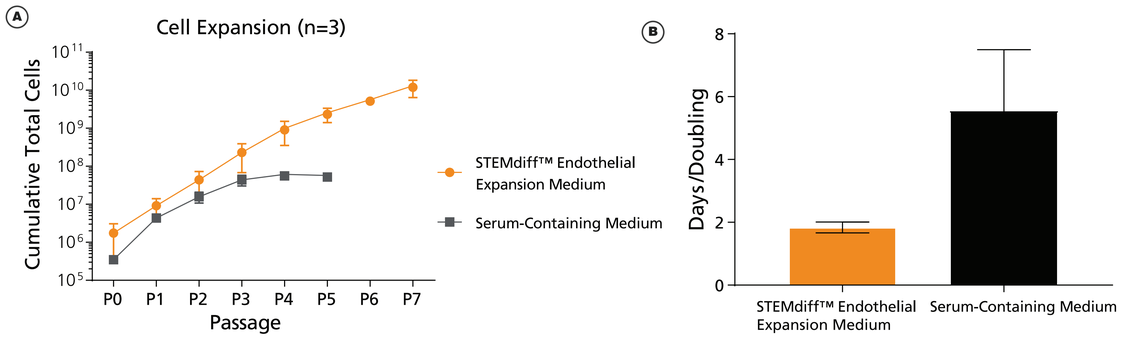

FIGURE 2. **(A)** Cell Expansion and **(B)** Doubling Rate of ECs Derived from iPS Cells (1C) in STEMdiff™ Endothelial Expansion Medium or Commercially Available Serum-Containing Medium

The average fold increase per passage over 5 passages for ECs derived from human iPS cells (1C) was ~4.3. The doubling time for human iPS-derived ECs was 1.8 per passage **(B)**.

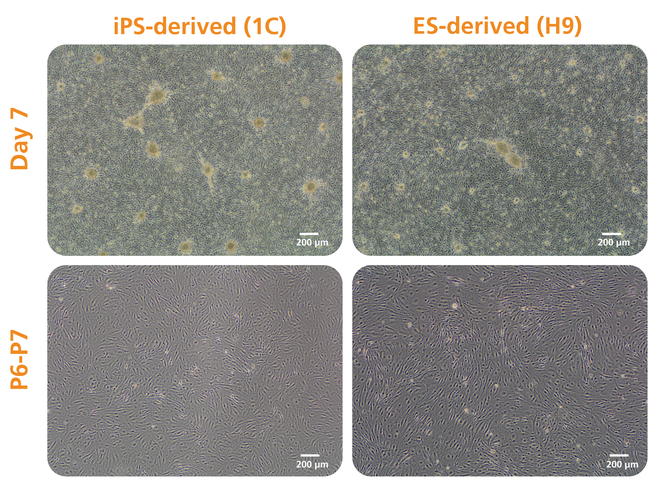

FIGURE 3. Representative Images of hPSCs Differentiating into ECs (1C and H9)

Human iPS (1C) and ES (H9) cells were vascularly induced using STEMdiff™ Vascular Induction Medium for 4 days. ES- and iPS-derived ECs were expanded in STEMdiff™ Endothelial Expansion Medium for 6 - 7 passages (P6 - P7) and showed typical endothelial morphology.

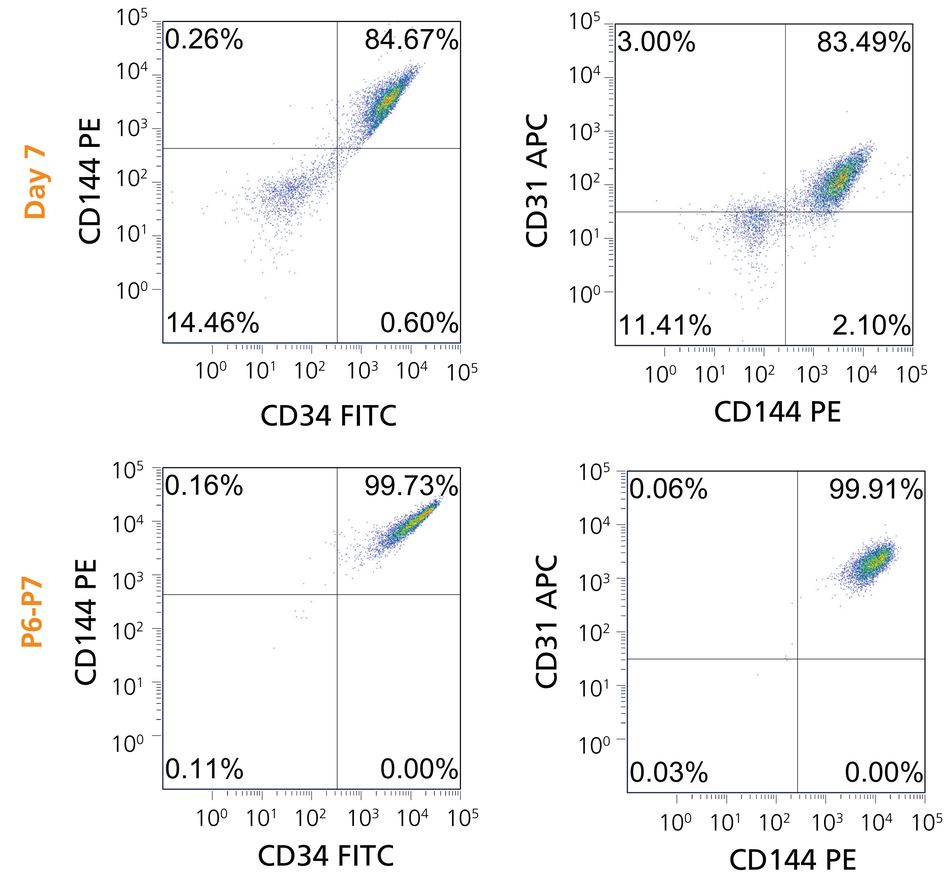

FIGURE 4. Representative Flow Cytometric Analysis of iPS-derived (1C) ECs on Day 7 and between Passages P6 - P7

On day 7 of hPSC-EC derivation, the cells express high levels of CD34, CD31, and CD144. Without any separation method, the cells were replated on ACF Attachment Substrate in STEMdiff™ Endothelial Expansion Medium for several passages. ECs maintained a high level of expression for these markers between passages P6 and P7.

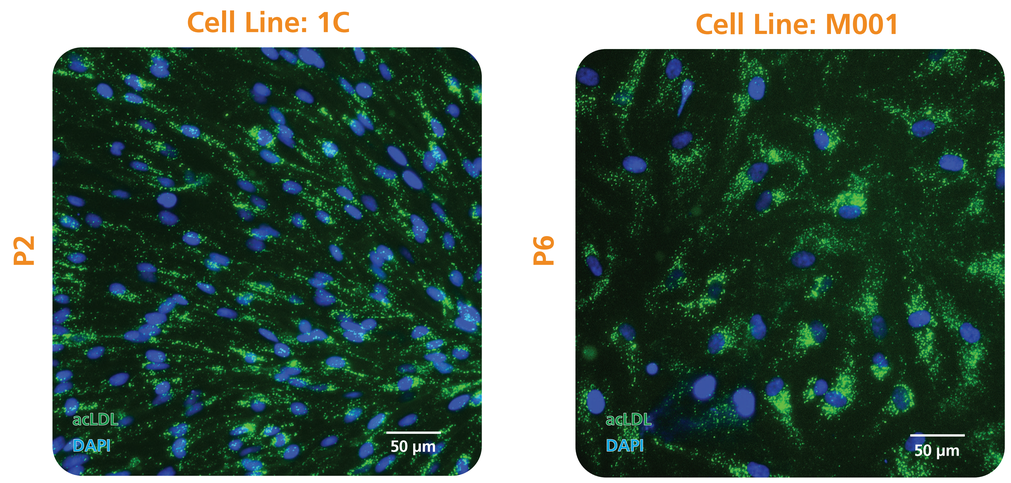

FIGURE 4. Functional Assay of hPSC-derived ECs

hPSC-derived endothelial cells are functionally active and able to incorporate acetylated LDL at early (P2) and late (P6) passage when cultured in STEMdiff™ Endothelial Expansion Medium.

Summary

- We have developed a workflow to efficiently derive (in ~7-10 days) and expand ECs from human ES and iPS cells in culture using xeno-free media, attachment substrate, and dissociation reagent.
- It is possible to obtain a high percentage of ECs at day 7 and enrich for CD34$^+$ cells without any separation methods.
- Human ES/iPS cell-derived ECs can be expanded long-term in STEMdiff™ Endothelial Expansion Medium.
- Human ES/iPS cell-derived ECs express a high level of endothelial markers (CD34, CD144, and CD31), maintaining a stable phenotype for > 6 passages in STEMdiff™ Endothelial Expansion Medium.
- Studies are underway to characterize other human ES and iPS cell lines at their transcriptomic level.