Defined and Xeno-Free Medium for Reprogramming Blood-Derived CD34+ Cells or Erythroid Cells

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Introduction

Peripheral blood (PB) is the second most commonly used primary cell source, after skin, for generating human induced pluripotent stem cells (hiPSCs), due to the minimally invasive procedure for sample collection. Numerous blood cell types have been reprogrammed such as hematopoietic stem and progenitor cells (HSPCs), T- and B-cells, erythroid precursors, and endothelial progenitor cells. While reprogramming efficiency is high in these cell types, the cells are often rare in PB and expansion is usually necessary to generate sufficient cell numbers for reprogramming. In this study, we developed an optimized, defined, feeder-free, and xeno-free reprogramming medium, ReproTeSR™, for use with CD34+ and erythroid cells expanded *in vitro* from PB. Together with EasySep™ CD34+ positive cell separation, StemSpan™ media and supplements for CD34+ and erythroid cell expansion, a workflow consisting of defined culture conditions for the isolation, expansion, and reprogramming of blood-derived cells can be established.

Materials & Methods

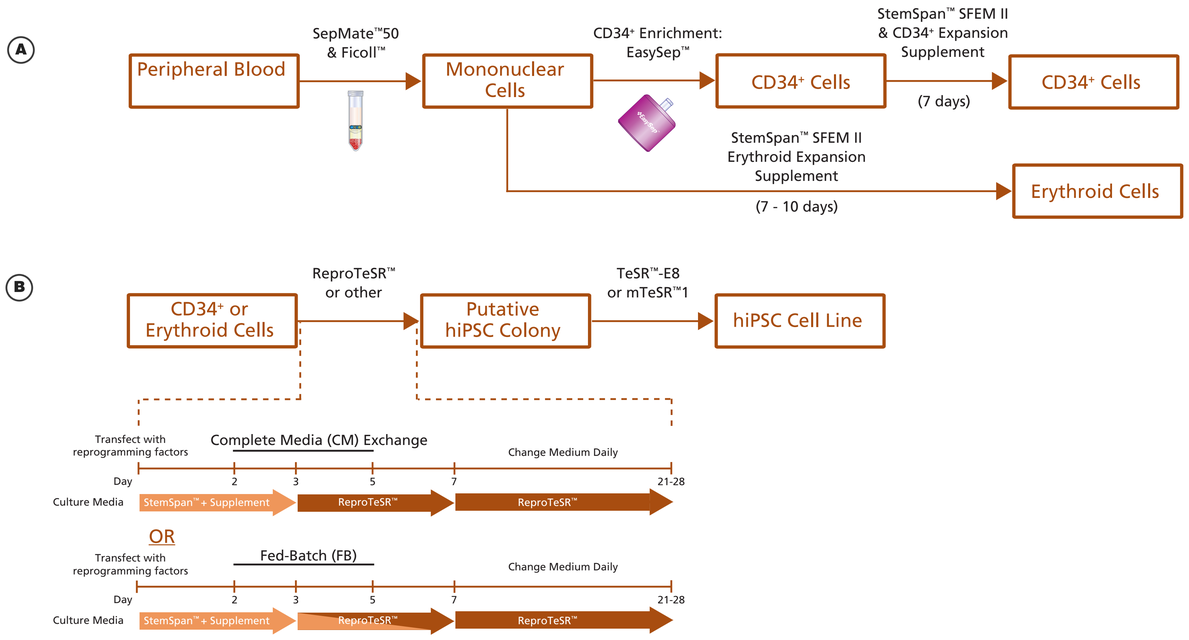

Figure 1. Workflow for the expansion and reprogramming of blood cells, followed by maintenance and differentiation of iPSC lines. **A)** Mononuclear cells (MNCs) were isolated from PB by fractionation over a Ficoll™ density gradient in SepMate™-50 (Catalog #15450) tubes. CD34+ cells were then enriched by immunomagnetic separation using EasySep™ CD34+ Positive Selection Kit (Catalog #18056), and expanded for 7 days in StemSpan™ SFEM II Medium (Catalog #09605) with CD34+ Expansion Supplement (Catalog #02691). Alternatively, erythroid precursor cells were generated by culturing PB MNCs for 7 - 10 days in StemSpan™ SFEM II with Erythroid Expansion Supplement (Catalog #02692). **B)** Culture-expanded CD34+ cells or erythroid precursor cells were then transfected with episomal vectors containing OCT4, SOX2, KLF4, and L-MYC, LIN28, and the cells immediately transferred to Matrigel®-coated plates at 30,000 cells/cm^2. Cells were cultured in StemSpan™ SFEM II Medium with CD34+ Expansion Supplement for 3 days following transfection, with daily media changes where all non-adherent cells were recovered by centrifugation and returned to the culture. Starting 3 days after transfection, media was changed to either ReproTeSR™ Medium, TeSR™-E7™ Medium (Catalog #05910), or hES medium (DMEM/F12, 20% KOSR, 10ng bFGF, L-Glutamine, NEAA, 2-ME) using 2 alternative feeding schedules. In the complete medium (CM) exchange method,on days 2, 3, and 5 all non-adherent cells were recovered by centrifugation and returned to the culture with fresh media. In the fed-batch (FB) method, 1mL of fresh medium is supplemented directly into the culture on days 3, 5, and 7. From day 7 onwards, reprogramming medium is replaced daily, until day 21 to 28, when colonies with iPSC morphology were identified by microscopic examination and counted. Selected colonies were transferred manually to Matrigel®-coated plates and cultured in mTeSR™1 Medium (Catalog #05850) or TeSR™-E8™ Medium (Catalog #05940) for further propagation of hiPSC lines.

Results

TABLE 1: Enrichment and expansion of CD34+ cells from human peripheral blood

	% CD34+ Cells After Enrichment	TNC Fold Expansion	CD34+ Cell Fold Expansion	% CD34+ Cells After Expansion	Number of CD34+ Cells After Expansion per Initial 100 mL PB
Mean (n = 6)	56	18	19	60	1.3 x 10^6
95% CL	39 - 74	12 - 25	15 - 22	47 - 63	0.1 - 2.5 x 10^6

TABLE 2: Expansion of erythroid precursor cells from human peripheral blood

	% CD71+/GlyA+ Cells in PB MNCs	Total Number of Cells After Expansion	% CD71+/GlyA+ Cells After Expansion	Number of CD71+/GlyA+ Cells After Expansion per Initial 10 mL PB
Mean (n = 7)	0.7%	3.3 x 10^6	82	2.0 x 10^6
95% CL	0.23 - 1.17	2.0 - 4.6 x 10^6	72 - 92	1.5 - 4.0 x 10^6

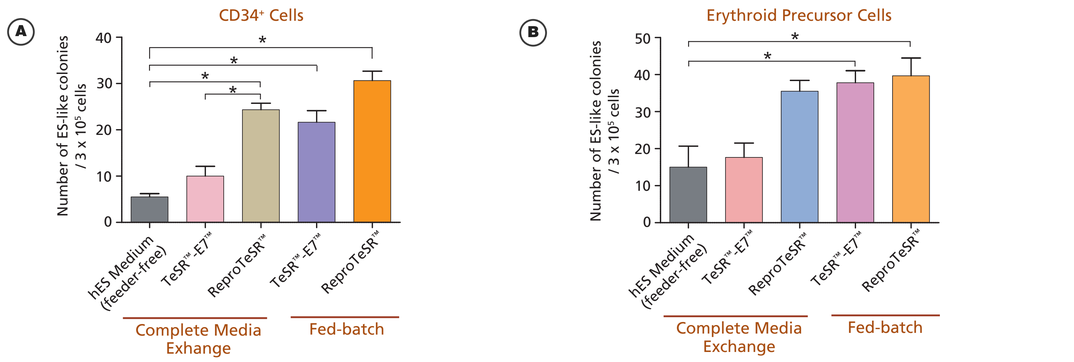

Figure 2. ReproTeSR™ Medium yields improved feeder-free reprogramming efficiency over standard hES medium. **A)** Reprogramming of culture-expanded CD34+ cells in feeder-free conditions using the complete media (CM) exchange method yeilds ~6-fold more iPSC colonies in ReproTeSR™ Medium compared to that in hES medium, and ~3-fold more compared to that in TeSR™-E7™ Medium. Applying a fed-batch (FB) method further enhanced the reprogramming efficiency in TeSR™-E7™ Medium or ReproTeSR™ Medium (mean ± SEM; n = 3; *p<0.05). **B)** Culture-expanded erythroid precursor cells showed improved feeder-free reprogramming efficiency in ReproTeSR™ Medium over hES medium or TeSR™-E7™ Medium by ~3-fold and ~2-fold, respectively, using the complete media exchange method. Applying a fed-batch method enhanced the reprogramming efficiency of both TeSR™-E7™ Medium and ReproTeSR™ Medium (mean ± SEM; n = 3; *p<0.05).

TABLE 3: Reprogramming efficiencies of CD34+ and erythroid precursor cells in ReproTeSR™ Medium

Cell Type	Total Number of Cells After Expansion per 10 mL blood	Reprogramming Efficiency	Number of Colonies per 3 x 10^5 cells plated	Number of Colonies per 10 mL blood
CD34+ Cells	3.6 x 10^5 ± 3.4 x 10^5	0.010 ± 0.002	29 ± 5	38
Erythroid Precursor Cells	3.3 x 10^6 ± 0.5 x 10^6	0.013 ± 0.003	39 ± 8	520

Mean ± standard deviation
CD34+ cells (n = 5)
Erythroid precursor cells (n = 3)

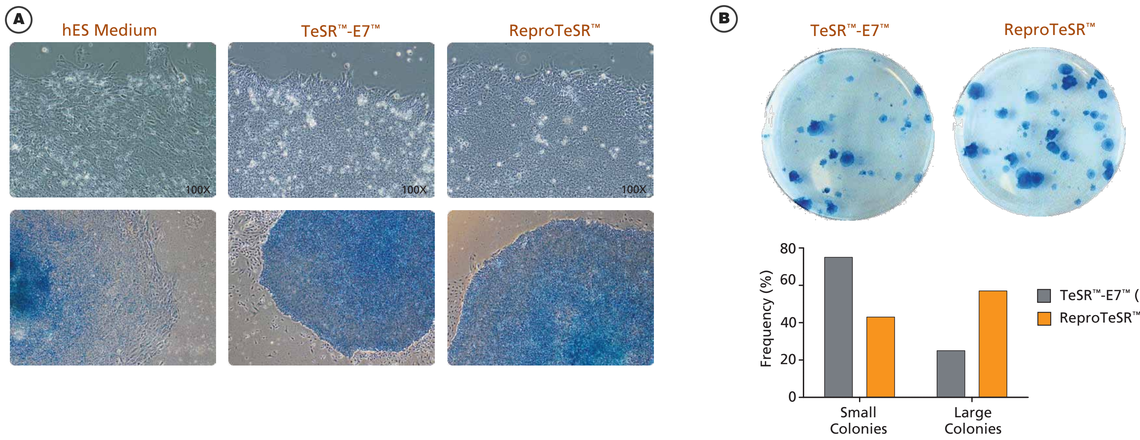

Figure 3. iPSC colonies derived in ReproTeSR™ Medium under feeder-free conditions show superior colony morphology and larger size. **A)** iPSC colonies derived from cultured-expanded CD34+ cells and reprogrammed in TeSR™-E7™ Medium or ReproTeSR™ Medium exhibited more defined borders, compact morphology, uniform alkaline phosphatase expression (blue), and reduced differentiation compared to hES medium. **B)** Reprogramming in ReproTeSR™ Medium yielded a greater proportion of large iPSC colonies by day 21 compared to TeSR™-E7™ Medium. Colonies derived in hES medium in feeder-free conditions were not measured due to poor morphology of the colonies.

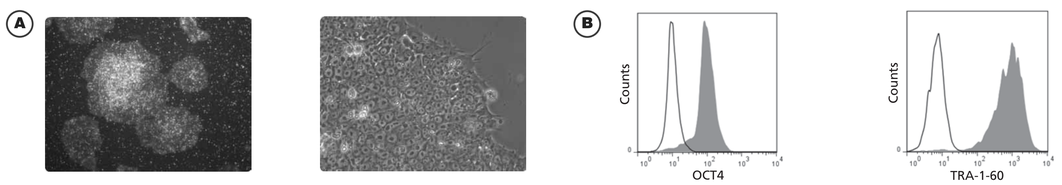

Figure 4. iPSCs derived in ReproTeSR™ Medium can be readily subcultured in mTeSR™1 Medium and express pluripotent markers. **A)** iPSC lines derived in ReproTeSR™ Medium and subcultured in mTeSR™1 Medium on Matrigel® displayed typical iPSC-like morphology with defined colony borders and high nuclear-to-cytoplasmic ratio. **B)** Flow cytometry analysis of iPSC lines derived in ReproTeSR™ Medium showed high percentage of cells expressing the pluripotency associated markers OCT4 and TRA-1-60.

Conclusions

- Peripheral blood-derived CD34+ or erythroid cells can be efficiently expanded using StemSpan™ SFEM II with CD34 Expansion Supplement or Erythroid Expansion Supplement, respectively.
- Reprogramming in feeder-free conditions is higher in TeSR™-E7™ Medium or ReproTeSR™ Medium compared to hES medium. This was further enhanced using a fed-batch feeding schedule within the first week of iPSC induction.
- ReproTeSR™ Medium facilitates the generation of large and well-defined iPSC colonies which can be identified for picking by day 21.
- iPSCs derived in ReproTeSR™ Medium are easily subcultured in mTeSR™1 Medium and express markers associated with pluripotency.