STEMdiff™ APEL™ is an Animal Component-Free Medium Which Supports Multi-Lineage Differentiation of Human Pluripotent Stem Cells

Jennifer Antonchuk[1], Michael J. Riedel[1], Mark Fairey[1], Simon Hilcove[1], Andrew Elefanty[2], Elizabeth Ng[2], Terry E. Thomas[1], Allen C. Eaves[1,3] and Sharon A. Louis[1]

[1] STEMCELL Technologies Inc, Vancouver, BC, Canada
[2] Monash Immunology and Stem Cell Laboratories, Melbourne, Australia
[3] Terry Fox Laboratories, BC Cancer Agency, Vancouver, BC, Canada

Introduction

Human pluripotent stem cells (hPSCs), including embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC), are characterized by their potential to differentiate into any somatic cell lineage. A diverse set of multi-step directed differentiation protocols have been described to induce differentiation to desired cell types, using a variety of media formulations, in embryoid body (EB)-based or adherent cell-based cultures. We have developed STEMdiff™ APEL™ Medium, a fully defined, animal component-free medium based on the publication of Ng *et al.*[1], which can be used as a base medium for differentiation of hPSCs to multiple lineages, in EB or adherent cell platforms. Here we demonstrate the utility of STEMdiff™ APEL™ Medium as a base media for cardiomyocyte, definitive endoderm or hematopoietic differentiation using cytokine combinations from the literature.

Materials & Methods

In this study, we used STEMdiff™ APEL™ Medium supplemented with cytokine combinations from the literature[1-4] to direct differentiation to cardiomyocyte, definitive endoderm, or hematopoietic lineages. In all cases input cells were maintained for at least 10 passages in mTeSR™1 prior to differentiation.

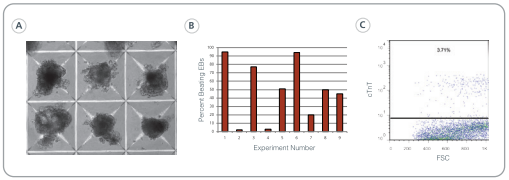
FIGURE 1: STEMdiff™ APEL™ Medium is an animal component-free medium for differentiation of human pluripotent stem cells

Cardiomyocyte differentiation was performed by making EBs in AggreWell™400, and then culturing the EBs in the AggreWell™ plate in STEMdiff™ APEL™ Medium with cytokine combinations based on the publication of Yang *et al.*[2]. Induction of hPSCs to the **definitive endoderm** lineage was carried out using an adherent cell based system, where cells were cultured on Matrigel™, and STEMdiff™ APEL™ Medium was supplemented with cytokines based on the publication of Rezania *et al.*[3]. **Hematopoietic** differentiation was carried out using an adherent cell based system, where cells were cultured on Matrigel™ and STEMdiff™ APEL™ Medium supplemented with cytokine combinations based on the publications of Ng *et al.*[1] (days 1-4) and Chadwick *et al.*[4] (days 5-13).

Results & Discussion

CARDIOMYOCYTE DIFFERENTIATION

FIGURE 2: Differentiation of hPSCs into cardiomyocytes using an AggreWell™400 EB-based protocol with STEMdiff™ APEL™ Medium

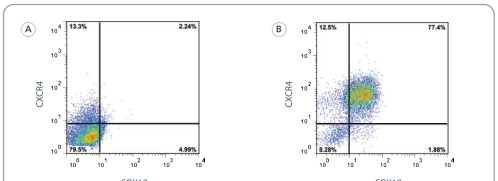


PSCs were differentiated into cardiomyocytes in STEMdiff™ APEL™ Medium supplemented with cytokines as described. **(A)** EBs in AggreWell™400 plates after 13 days of differentiation. Transparent areas at the edges of the EBs tend to show beating. **(B)** Beating EBs were counted on Day 16 of the culture, and varied between 5% and 95% of total EBs (n=9). **(C)** Expression of cTnT was measured on day 16 by flow cytometry. Shown is a representative example of cTnT positive cells, from directed differentiation of H9 hESCs.

As shown in Figure 2, up to 95% of EBs were found to be beating on day 16 of the described protocol. The percentage of cells that were cardiac troponin T (cTnT) positive averaged 3.2% (± 2.3% SD), indicating that a minority of cells within each beating EB were true cardiomyocytes. This example demonstrates how STEMdiff™ APEL™ Medium can support differentiation of mTeSR™1-grown cells into beating cardiomyocytes.

DEFINITIVE ENDODERM INDUCTION

FIGURE 3: Differentiation of hPSCs into definitive endoderm with STEMdiff™ APEL™ Medium

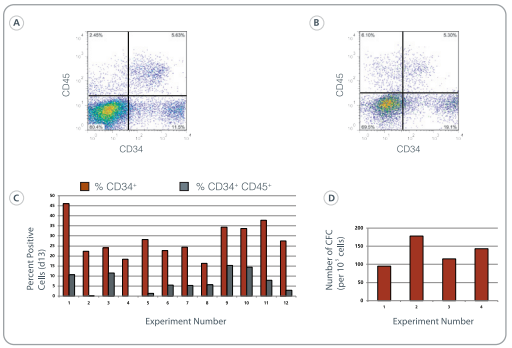


H9 ES cells were differentiated based on the protocol described. On day 4, cells were highly positive for CXCR4 and Sox17, which together mark definitive endoderm (**A:** isotype control **B:** differentiated culture).

As shown in Figure 3, the majority of cells co-expressed CXCR4 and Sox17 after 4 days in STEMdiff™ APEL™ Medium supplemented with endoderm-inducing cytokines. An average of 69.53 ± 9.1% CXCR4$^+$Sox17$^+$ cells were obtained using this procedure (n=2, each in triplicate). The frequency of cells expressing CXCR4 (>90%) is consistent with data shown in Rezania *et al.*[3] indicating that STEMdiff™ APEL™ Medium can successfully replace other FBS- or BSA-containing basal media alternatives for definitive endoderm induction.

HEMATOPOIETIC DIFFERENTIATION

FIGURE 4: Differentiation of hPSCs into hematopoietic cells with STEMdiff™ APEL™ Medium



Expression of hematopoietic markers CD34 and CD45 in **(A)** iPS4D1 and **(B)** H9 cells after 13 days of directed hematopoietic differentiation. **(C)** Between 15% and 45% of cells expressed CD34, and an average of 7.3% co-expressed CD45 (n=12). **(D)** CFCs were detected at a frequency of 133 ± 36 (mean ± SD; n=4) per 10^5 cells plated.

As shown in Figure 4, 28.0% ± 8.6% (mean ± SD; n=12) of cells expressed CD34, and 7.3% ± 5.1 were CD34$^+$CD45$^+$. This was further correlated with the presence of colony-forming cells at a frequency of approximately 1 in 1,000 cells (Figure 4D). This example demonstrates the utility of STEMdiff™ APEL™ Medium as a serum-free, animal component-free alternative for use in an established hematopoietic differentiation protocol.

Summary

STEMdiff™ APEL™ Medium is a serum-free, animal component-free, and growth factor-free medium for directed differentiation of hPSCs to a variety of downstream lineages. Here we have shown directed differentiation of hES and hiPS cell lines previously maintained on mTeSR™1, using STEMdiff™ APEL™ Medium supplemented with lineage-specific cytokines in EB- or adherent cell-based approaches. Using the right combination and timing of cytokines it is likely that hPSCs can differentiate to many somatic cell lineage in STEMdiff™ APEL™ Medium.

REFERENCES

1. Ng ES, *et al.* Nat. Protocols 3: 768-776, 2008.
2. Yang L *et al.* Nature 453: 524-528, 2008.
3. Rezania A, *et al.* Diabetes 60: 239-247, 2011.
4. Chadwick K, *et al.* Blood 102: 906-915, 2003.

Download the poster with your smartphone
SCAN ME

STEMCELL™ TECHNOLOGIES