Modulating Growth Factor Addition to Essential 6™ Media for a Complete Xeno-free Media From Fibroblast Culture to iPSC Generation and Expansion

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ABSTRACT

The generation of induced pluripotent stem cells (iPSCs) from somatic cells has the potential to revolutionize cell-based therapeutic applications. Current methods require multiple media systems for various stages of reprogramming. An ideal solution would be a media system that can be used from the original patient sample harvest to expansion of the resulting iPSC clones.

Essential 8™ media has emerged as an easy, simple and cost effective media system for the expansion of pluripotent ESC and iPSCs. Here, we use the basal version without growth factors, termed Essential 6™ media as the base media and optimize the addition of the two key growth factors, bFGF and TGFβ, at various stages during reprogramming. Our results suggest that unlike other xeno-free media systems, Essential 8™ media supports efficient transduction via Sendai virus without toxicity. Furthermore, optimal timing of TGFβ removal a week after transduction supported efficient reprogramming resulting in iPSC colonies that were subsequently expanded on recombinant human vitronectin and Essential 8™ media. Characterization studies indicated a normal karyotype, pluripotency marker expression, and in vitro differentiation into all three lineages after multiple passages. While use of the Essential 8™ media system has been reported for episomal reprogramming, its optimization in other reprogramming methods and workflows enables a modular, easy to use, feeder-free, xeno-free system.

INTRODUCTION

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed adult cells which exhibit a pluripotent stem cell-like state similar to embryonic stem cells (ESCs)[1]. Although these cells are not known to exist in the human body they share significant similarities to ESCs. Therefore, iPSCs serve as an important new source of cells for drug discovery, cell therapy, and basic research. There are multiple methods to generate iPSCs. The CytoTune™–iPS Reprogramming System uses vectors based on replication-incompetent Sendai virus (SeV) to safely and effectively deliver and express key genetic factors necessary for reprogramming somatic cells into iPSCs. The current reprogramming protocol incorporates multiple media systems and growth substrates that have animal components. Essential 8™ is a xeno-free medium that has been shown to support multiple ESC and iPSC lines in a feeder-free environment[2,3]. Here we demonstrate that Essential 8™, Essential 6™ (basal medium without growth factors), and recombinant human vitronectin can be incorporated into the CytoTune™ protocol to generate and expand iPSCs; thus enabling a modular, easy to use, feeder-free xeno-free system.

MATERIALS AND METHODS

All reagents were purchased from Life Technologies™, unless otherwise noted.

Cell culture: CytoTune™-generated iPSC lines were maintained on recombinant human vitronectin (Cat# A14701SA) using Essential 8™ media (Cat# A14666SA). At ~70-80% confluency cultures were passaged using 0.5mM EDTA (Cat# AM9260G).

Reprogramming: Human neonatal foreskin fibroblast cells (ATCC, Cat# CRL2522) were cultured and transduced using CytoTune™ Sendai vectors (MOI = 5) and Essential 8™. Six days post transduction cells were cultured in Essential 6™ (Cat# A1516401) + bFGF (100ng/mL) (Cat# PHG0261). Transduced cells were harvested using 0.5mM EDTA and seeded onto recombinant human vitronectin. At ~21 days post transduction iPSC colonies were manually picked and transferred to vitronectin-coated dishes and Essential 8™.

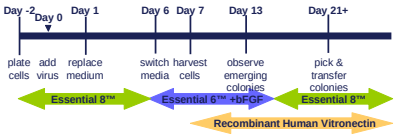
Pluripotent characterization: Pluripotency was determined by live immunostaining using directly conjugated antibodies to surface markers: SSEA-4 (Cat# SSEA421), Tra-1-60 (Cat# 411000).

Cytogenetic characterization: G-band karyotypes were prepared and analyzed for each CytoTune™ generated iPSC line.

Differentiation characterization: Embryoid bodies were formed from the CytoTune-generated iPSC lines and subjected to undirected differentiation on either Geltrex- or recombinant human vitronectin- coated plates for 21 days. Cultures were fixed and stained with the appropriate antibodies for endoderm, mesoderm, and ectoderm markers.

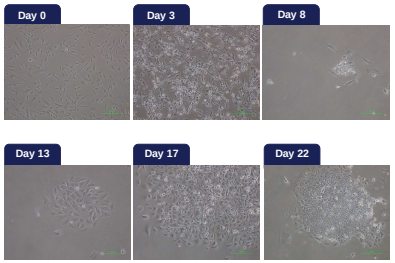
RESULTS

Figure 1. CytoTune™ reprogramming protocol can be modified to use xeno-free media and substrate



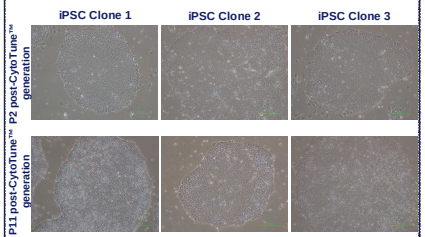
Chemically-defined Essential 8™ media was employed for BJ fibroblast culture and transduction with the CytoTune™ Sendai reprogramming vectors. Following transduction the media was switched to Essential 6™ + bFGF in order to promote stem cell growth. Transduced cells were harvested and transferred to vitronectin-coated dishes. At ~ 14d post transduction the cultures were switched to Essential 8™. By ~ 21d mature iPSC colonies were picked and transferred to fresh vitronectin-coated dishes for expansion.

Figure 2. Representative images collected during CytoTune™ reprogramming using Essential 8™, Essential 6™, and vitronectin



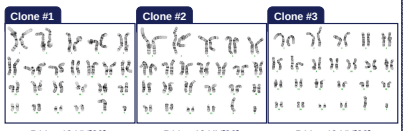
BJ fibroblasts were seeded on tissue culture polystyrene with Essential 8™. At Day 0 CytoTune™ vectors were added at an MOI = 5. Significant cytotoxicity was observed by Day 3. By Day 8 (24h after harvesting transduced cells) small clusters of cells were present. On Day 22 iPSC colonies were picked and expanded.

Figure 3. Morphology of iPSC clones generated using xeno-free media and substrate



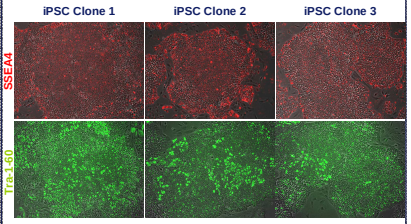
A total of 3 iPSC clones were generated from $5x10^4$ transduced cells that were harvested on Day 7 and seeded onto vitronectin-coated plates (reprogramming efficiency = 0.01%). All three clones appear to have typical stem cell colony morphology (smooth edge, high nucleus-to-cytoplasm ratio) at early and later passages.

Figure 4. iPSC clones generated using xeno-free media and substrate demonstrate normal karyotpyes



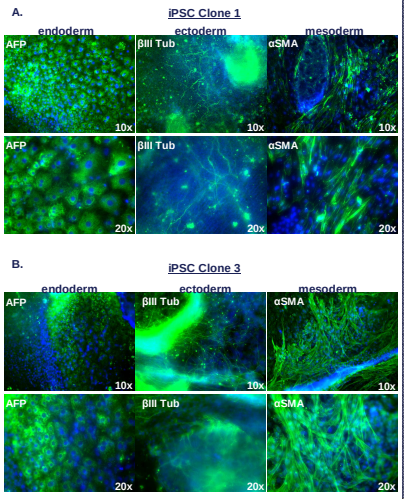
All three iPSC clones demonstrated normal human karyotypes after 11 passages on recombinant human vitronectin. Cytogenetic analysis on twenty G-banded metaphase cells from each iPSC clone was performed by Cell Line Genetics, Inc.

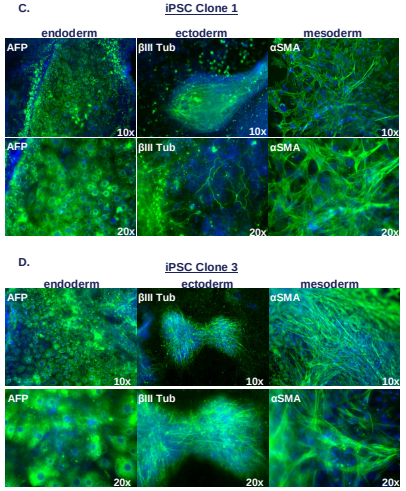
Figure 5. iPSC clones generated using xeno-free media and substrate maintain pluripotency



All three iPSC clones expressed SSEA4 and Tra-1-60 after 11 passages on recombinant vitronectin.

Figure 6. iPSC clones generated using xeno-free media and substrate demonstrate differentiation along all three germ layers





iPSC clones demonstrated differentiation into cell types from all three germ layers (endoderm, ectoderm, and mesoderm) after 12 passages on recombinant vitronectin. iPSC Clone 1 was differentiated in Essential 6™ on A) vitronectin (1µg/cm²) or C) 1:100 (v/v) Geltrex™. iPSC Clone 3 was differentiated in Essential 6™ on B) vitronectin (1µg/cm²) or D) 1:100 (v/v) Geltrex™.

CONCLUSIONS

- Essential 8™, Essential 6™, and recombinant human vitronectin can be incorporated into the current CytoTune™ reprogramming protocol to generate iPSC colonies at an efficiency of 0.01%
- iPSC colonies generated under these xeno-free conditions demonstrate:
  - normal morphology and normal karyotype after extended culture
  - maintenance of pluripotency marker expression after extended culture
  - ability to differentiate along all three germ lineages following extended culture
- Reprogramming efficiency can likely be increased using additional chemical additives (e.g., sodium butyrate)

REFERENCES

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