Reprogramming of somatic cells to induced-pluripotent stem cells in TeSR™-E7™, a feeder-free, defined, and low-protein medium

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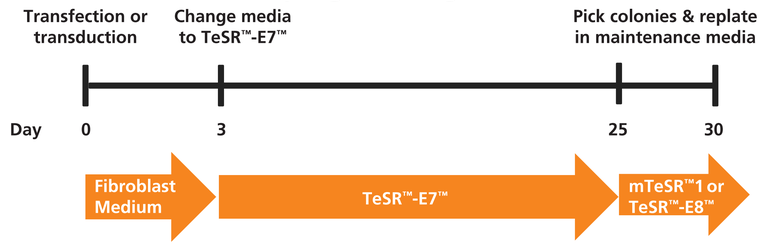
Introduction

The discovery that nuclear reprogramming to pluripotent stem cells (PSCs) can be achieved by the exogenous expression of a small number of transcription factors has opened up new possibilities in regenerative medicine. The resulting cells, termed induced-pluripotent stem (iPS) cells, have been generated by exogenous expression of the reprogramming transcription factors via multiple delivery systems and media conditions, with a general movement towards more defined, clinically relevant systems. While iPS cells were initially generated using stable integration of the reprogramming factor genes in retrovirus or lentivirus vectors, increasingly delivery systems are being developed as non-integrating, transient expression systems such as episomal DNA vectors or mRNA. In addition, defined and feeder-independent media conditions have been modified to avoid the introduction of undefined factors in the iPS cell derivation step. Recently, Chen et al. [1] published modifications of the TeSR™ medium formulation to create a simplified, low-protein medium for either feeder-free maintenance of human PSCs (termed E8) or reprogramming of fibroblasts into iPSCs (termed E7). Based on the E8 formulation, we have recently released TeSR™-E8™ Medium for maintenance of human PSCs, and have now developed a simplified low-protein defined medium for reprogramming, called TeSR™-E7™. Our data demonstrates that TeSR™-E7™ is highly efficient for feeder-free reprogramming and generation of iPS cell colonies which are homogeneous and can be unambiguously identified as embryonic stem (ES) cell-like colonies.

Methods

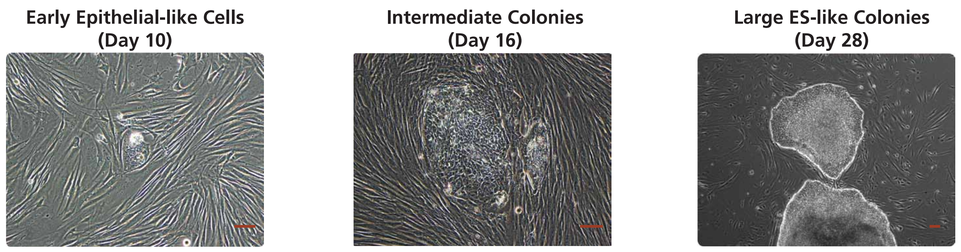
FIGURE 1. Schematic of fibroblast reprogramming to iPSCs in TeSR™-E7™



Adult primary dermal or neonatal fibroblasts were cultured in fibroblast medium (DMEM and 8% FBS, or other commercial medium) prior to reprogramming (day 0). To initiate reprogramming, 5 x 10^4 fibroblasts were transfected with an episomal vector containing the reprogramming factors and then plated on a 6-well plate pre-coated with either Matrigel™ (BD Biosciences) or Vitronectin-XF™ (STEMCELL Technologies, Catalog #07190). Three days post transfection the fibroblast medium was removed and replaced with TeSR™-E7™, which was used for the remainder of the induction phase of reprogramming with daily media changes. 3 - 4 weeks post-transfection (day 25 - 30), colonies were identified based on ES cell-like morphology, isolated manually, and subcultured in either mTeSR™1 or TeSR™-E8™ on Matrigel™ or Vitronectin-XF™ pre-coated plates. For further maintenance, cells were passaged with Gentle Cell Dissociation Reagent (STEMCELL Technologies, Catalog #07174).

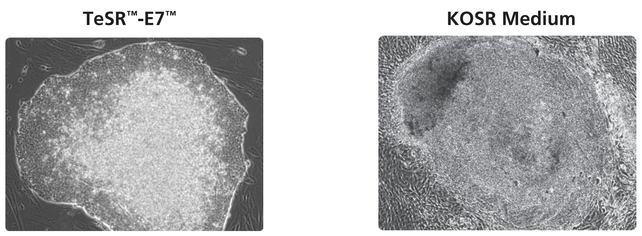
Results

FIGURE 2. Morphology of emerging iPSC colonies during the induction phase of reprogramming in TeSR™-E7™



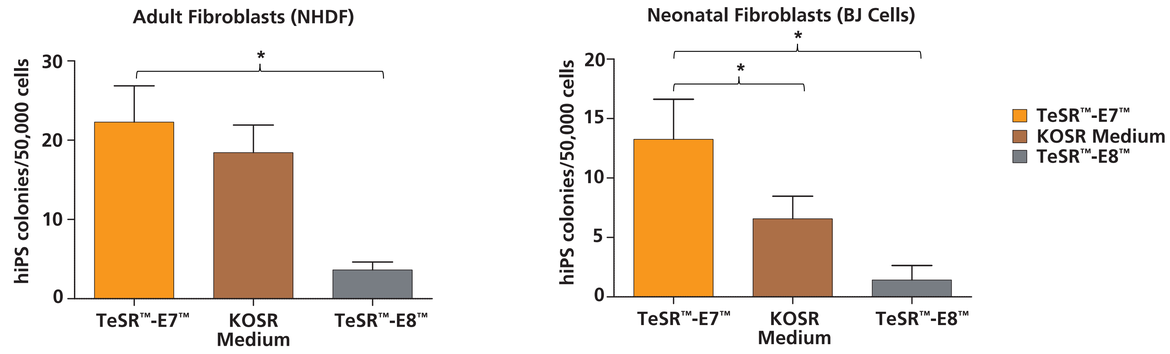
Emerging colonies were imaged during the induction phase of reprogramming in TeSR™-E7™ to monitor changes in morphology. Fibroblasts reprogrammed in TeSR™-E7™ start to exhibit mesenchymal-to-epithelial changes over the first 10 days of iPS cell induction, depicted by the conversion of a more spindle-like shape cellular phenotype to cells exhibiting compact association and characteristics. More notable cellular changes are observed after approximately 2 weeks (day 16) seen here as increased nuclear-to-cytoplasmic ratio and pronounced nucleoli. ES cell-like colonies typically arise between 3 and 4 weeks (day 28) in TeSR™-E7™ exhibiting distinct colony borders and compact colony morphology. Scale bars = 100 µm.

FIGURE 3. High quality iPS cell colony morphology in TeSR™-E7™



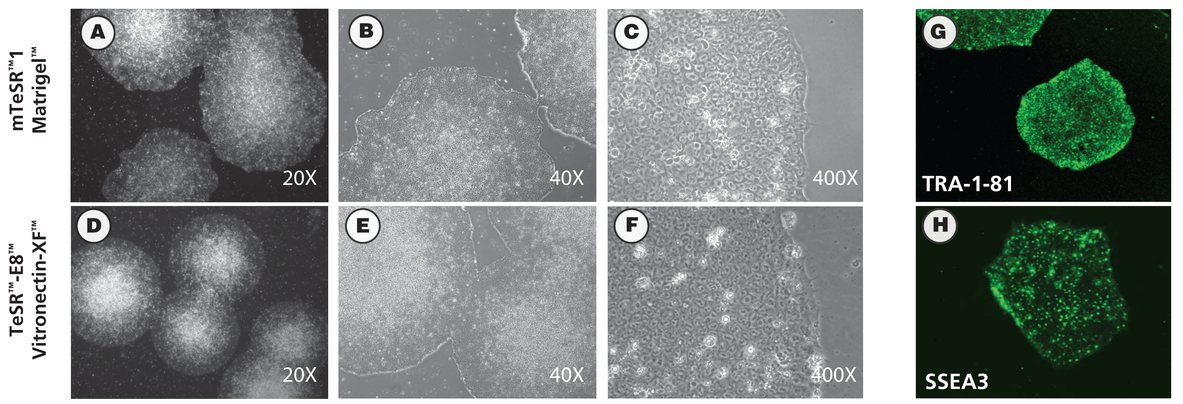
ES cell-like colonies generated during the induction phase in TeSR™-E7™ typically display improved morphology compared to those derived in KOSR Medium (DMEM/F12, 20%KOSR, 10 ng/ml bFGF). Emerging colonies in TeSR™-E7™ have more distinct borders and phase-bright dense centers, compact morphology. In addition, non-reprogrammed fibroblasts have slower growth rate, leading to less overgrowth of fibroblasts in TeSR™-E7™, which can obscure the colony in KOSR medium.

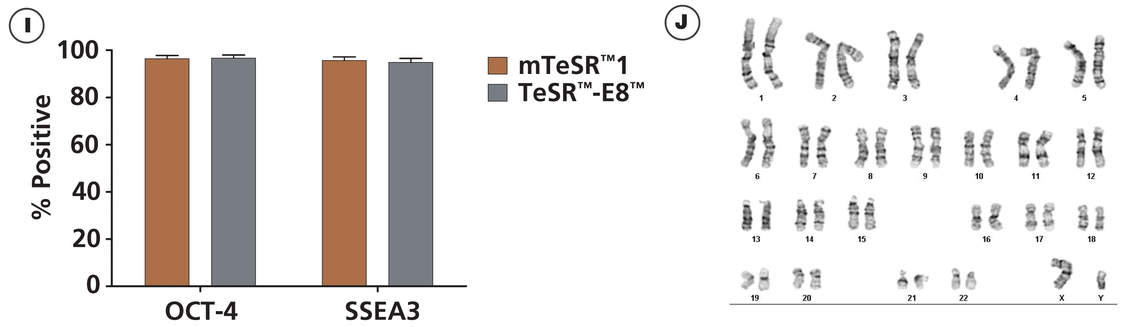
FIGURE 4. High reprogramming efficiency in TeSR™-E7™



Reprogramming efficiencies (number of colonies per well) in 2 fibroblast lines were compared using TeSR™-E7™ Reprogramming Medium, KOSR medium, and TeSR™-E8™ Maintenance Medium. TeSR™-E7™ reprogramming defined medium facilitated reprogramming fibroblasts into ES cell-like colonies with equivalent efficiency as undefined KOSR medium, and consistently 2 to 4-fold higher efficiency than the defined maintenance medium TeSR™-E8™. Data expressed as mean +/- SEM. *Denotes p value < 0.05.

FIGURE 5. iPS cells derived in TeSR™-E7™ can be subcultured in mTeSR™1 or TeSR™-E8™ without adaptation, express pluripotent markers, and exhibit normal karyotype





Induced-pluripotent stem cells were derived in TeSR™-E7™, manually selected and subcultured in either mTeSR™1 with Matrigel™ or TeSR™-E8™ with Vitronectin-XF™ for up to 10 passages. Cultures were easily adapted to either mTeSR™1 (A-C) or TeSR™-E8™ (D-F), and displayed excellent characteristic ES-like morphology with little differentiation (passage 6). Further analysis of cells cultured in either mTeSR™1 or TeSR™-E8™ showed high expression of pluripotent marker expression such as Tra-1-81, SSEA3, and Oct4 by both immunostaining (G and H) and flow cytometry (I; n = 2; ± SD) approaches, respectively. (J) Karyotype analyses of TeSR™-E7™ derived iPS cells showed no chromosomal anomalies at passage 6.

Summary

TeSR™-E7™ Reprogramming Medium

- **Feeder-Free:** derivation of iPS from fibroblasts without a feeder-layer
- **Simplified**: formulation is serum-free, xeno-free, and low-protein
- **High Quality**: emerging colonies have recognizable ES-like morphology and less background fibroblast overgrowth
- **Efficient:** generation of iPS colonies at frequencies similar to that of standard, un-defined system
- **Compatible:** colonies are easily sub-cultured into mTeSR™1 or TeSR™-E8™ defined maintenance systems
- **High Fidelity:** iPS cells express pluripotency markers and are karyotypically normal