A serum-free and feeder-free defined medium that enables weekend-free culture of undifferentiated human pluripotent stem cells

Min Lu[1], Naomi Guyette[1], Boris Greber[2] and Vi Chu[1]

[1]Merck Millipore, Billerica, MA, USA; [2]Max Planck Institute for Molecular Biomedicine, Münster, Germany

Abstract

Current media formulations used to culture undifferentiated pluripotent human ES and induced pluripotent stem (iPS) cells require medium replenishment every day and at least one medium exchange on the weekend. The costs in media, reagents and human resources become prohibitively expensive as increasing numbers of core labs and consortia are focused on generating diseased human iPS cell models. We have identified 11 essential components in a serum-free and feeder-free medium that enables weekend-free culture of undifferentiated human pluripotent stem cells and allows for medium exchanges every other day without compromising the morphology or long-term functionality of pluripotent stem cells. These 11 components include HSA, lowered FGF (10% of E8), TGFβ1 (25% of E8) and Activin A concentrations and small molecule inhibitors to suppress spontaneous differentiation. For the study, H9 human ES cells and 2 different clones of transgene-free human iPSCs were used. Transgene-free human iPSCs were generated by a Cre-excisable polycistronic lentiviral vector expressing the "stem cell cassette" (STEMCCA™ vector) comprised of all four transcription factors (OKSM) followed by exposure of the full reprogrammed iPSC to cell-permeable TAT-Cre recombinant protein. All three pluripotent cell lines, whether originally cultured in feeder-based or feeder-free cultures, could be easily and directly adapted to the 11-component medium without noticeable differences in cell vitality and cell health. Pluripotent cells maintained in the 11 component medium (>20 passages) expressed high levels (>90%) of pluripotency markers including SSEA4, OCT4, TRA-1-60 and TRA-1-81, possessed normal karyotype and were capable of differentiating into derivatives of all three germ layers *in vitro* and *in vivo*. Remarkably, H9 cells remained undifferentiated and exhibited minimal signs of spontaneous differentiation and could furthermore be serially passaged with a minimal 1 day per week medium exchange. While a 25-33% reduction in the number of attached colonies was observed, the colonies remained undifferentiated and proliferated at a similar apparent rate (i.e. 5-6 days between passages) to controlled cultures of regular medium exchanges. These unexpected results led us to determine the long-term effects of a more moderate feeding regiment of every other day combined with an absence of weekend media replacement. Pluripotent cells maintained in this moderate feeding regiment exhibited high cell health with minimal spontaneous differentiation; expressed high levels of pluripotency markers and possessed a normal karyotype. Current efforts are aimed at identifying the critical component(s) that confer this advantage and whether nutrient-sensitive signaling pathways that regulate energy metabolism may be involved.

PluriSTEM™ Human ES/iPS Cell Medium

- Small molecule-based defined feeder-free, serum-free medium for expansion of human pluripotent ES and iPS cells (>30 passages).
- Contains 11 essential components: lowered FGF (10% of E8), TGFβ1 (25% of E8), Activin A and small molecule inhibitors.
- **Eliminates the requirement to feed cells over the weekend. Take the weekend off.**
- **Eliminates the requirement of daily feeding. While daily feeding is optional, cells may also be transitioned to every other day (i.e. Monday, Wednesday, Friday medium exchanges).**
- Simple & easy transition to PluriSTEM™ medium from feeder-based and feeder-free culture systems. No period of low cell yield.
- Superior single cell culture, passage and selection of human pluripotent clones.
- **Superior freeze-thaw cell viability and recovery.**
- Supports suspension culture.
- **Each lot is rigorously quality control-tested for ability to sustain human ES/iPS for ≥ 3 passages. Available as Cat. No. SCM130.**

PluriSTEM™ medium performs comparably to competitor medium

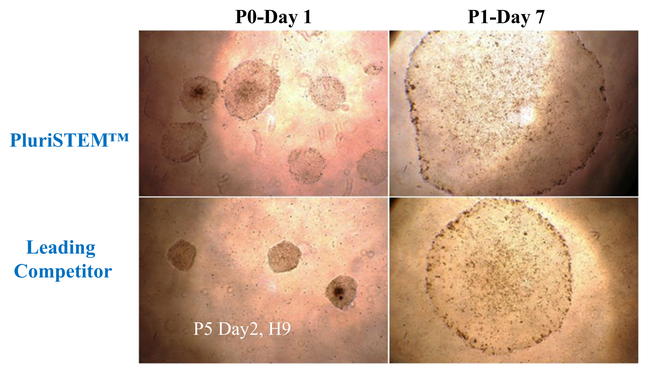
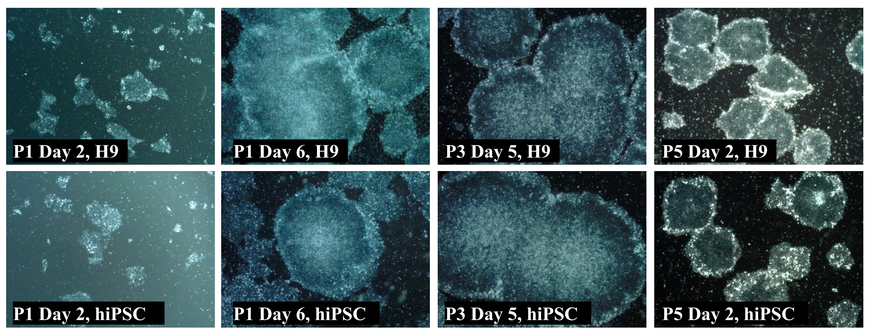
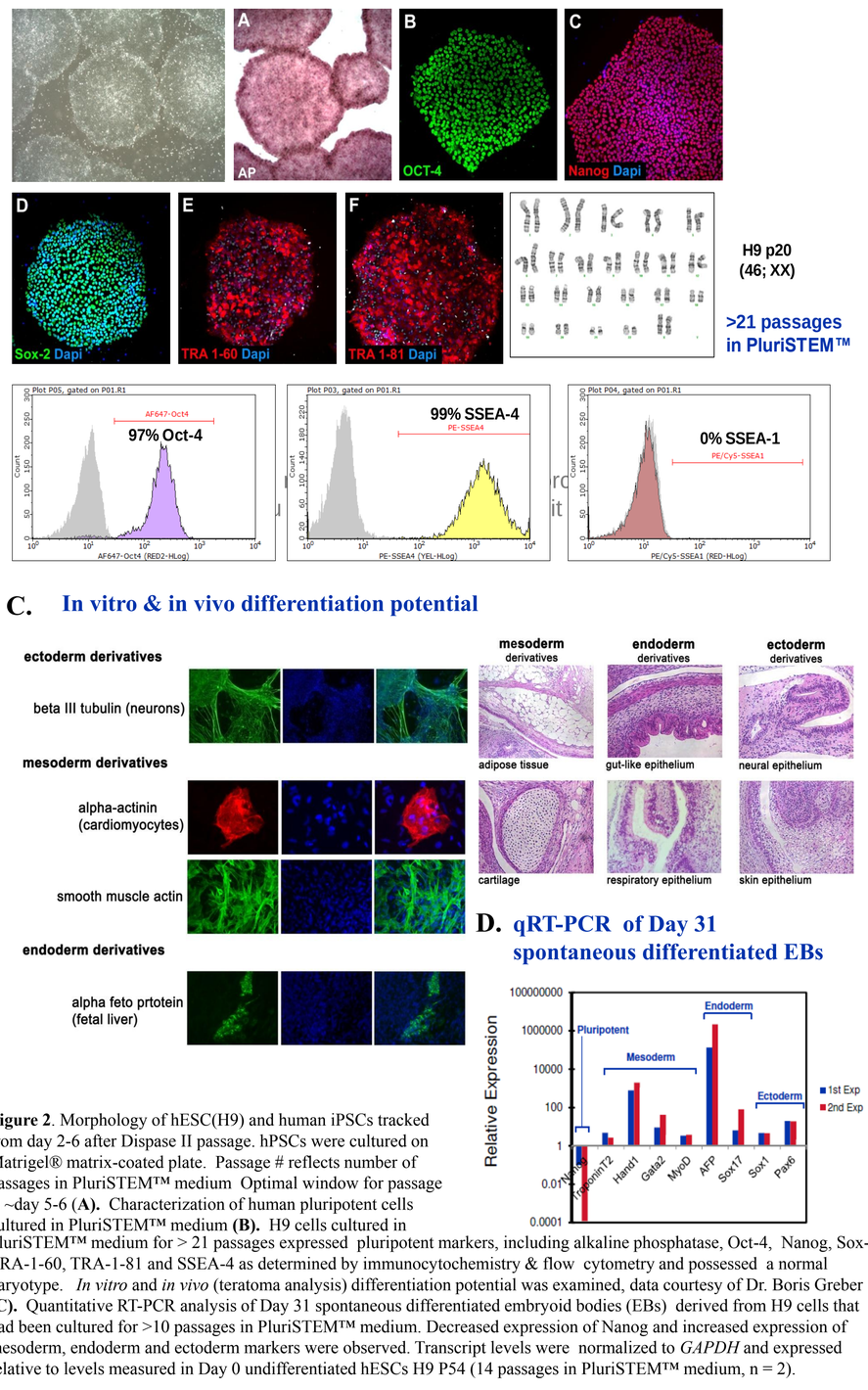


Figure 1. Human iPS cells generated using Cre-excisable STEMCCA™ vector (OKSM) were cultured in PluriSTEM™ and a leading competitor's medium on the same Matrigel® matrix-coated plate. More than one cell lines were tested and results were similar.

A. Morphology of hESCs and hiPSCs grown in PluriSTEM™ medium



B. PluriSTEM™ medium maintains hES and hiPS cells in pluripotent state



C. In vitro & in vivo differentiation potential

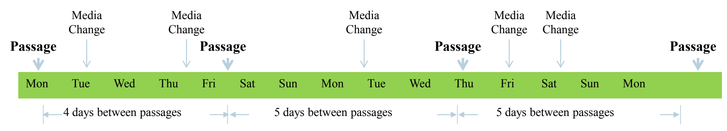
D. qRT-PCR of Day 31 spontaneous differentiated EBs

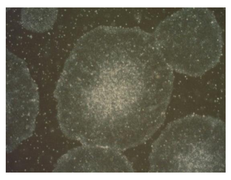
Figure 2. Morphology of hESC(H9) and human iPSCs tracked from day 2-6 after Dispase II passage. hPSCs were cultured on Matrigel® matrix-coated plate. Passage # reflects number of passages in PluriSTEM™ medium Optimal window for passage is ~day 5-6 (**A**). Characterization of human pluripotent cells cultured in PluriSTEM™ medium (**B**). H9 cells cultured in PluriSTEM™ medium for > 21 passages expressed pluripotent markers, including alkaline phosphatase, Oct-4, Nanog, Sox-2, TRA-1-60, TRA-1-81 and SSEA-4 as determined by immunocytochemistry & flow cytometry and possessed a normal karyotype. *In vitro* and *in vivo* (teratoma analysis) differentiation potential was examined, data courtesy of Dr. Boris Greber (**C**). Quantitative RT-PCR analysis of Day 31 spontaneous differentiated embryoid bodies (EBs) derived from H9 cells that had been cultured for >10 passages in PluriSTEM™ medium. Decreased expression of Nanog and increased expression of mesoderm, endoderm and ectoderm markers were observed. Transcript levels were normalized to *GAPDH* and expressed relative to levels measured in Day 0 undifferentiated hESCs H9 P54 (14 passages in PluriSTEM™ medium, n = 2).

Superior advantages provided only by PluriSTEM™ medium

(1) Weekend free & decreased media consumption

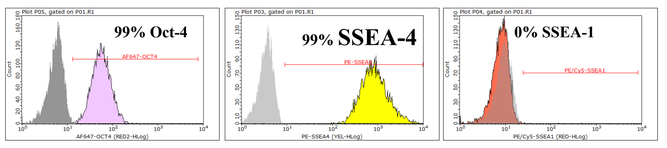
A. Every other day medium change and skip weekends



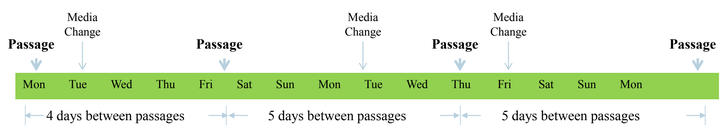


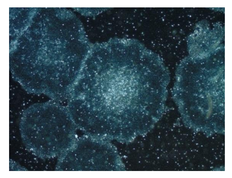
- **Media change one day after passage**
- **Every other day media exchange**
- **No weekend media exchange**
- **4 -5 days between passages**

H9 (p23 in PluriSTEM™ medium)
(P7 in moderate medium change)



B. Cells cultured in PluriSTEM™ medium can withstand high stress conditions (One medium change per passage)





- **Medium change one day after passage**
- **No medium change until next passage**
- **No weekend medium exchange**
- **4 -5 days between passages**

H9 (p23 in PluriSTEM™ medium)
(P8 in stressful condition)

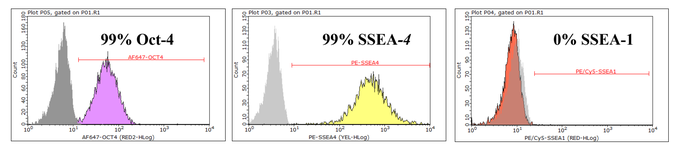
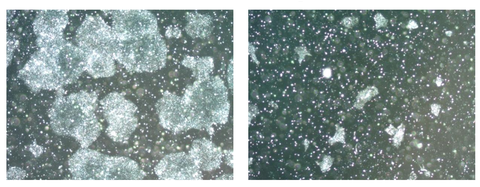


Figure 3. Schematic of 2 feeding schedules. H9 cells were cultured in PluriSTEM™ medium following the 2 feeding schedules (A – moderate, B - stressful)for 10 passages before subjected to flow cytometry. In both conditions, human pluripotent cells retained undifferentiated cell morphology, high cell health and expressed high levels of pluripotent markers, Oct4 and SSEA-4 and low levels of SSEA-1.

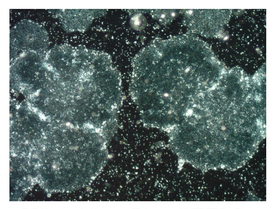
(2) Superior single cell culture & expansion in PluriSTEM™ medium



6 days after single cell plating

Figure 4. Single cells proliferate more rapidly in PluriSTEM™ medium relative to top competitor's medium. H9 hESCs (P22 in PluriSTEM™ medium) were dissociated into single cells and 100,000 cells/well were seeded into 6-well-plate. Rock inhibitor was added one hour before harvesting and 24 hrs post-plating. Cells in PluriSTEM™ medium formed large discrete colonies after 6 days and are ready to be passaged.

(3) Superior freeze-thaw viability with PluriSTEM™ Freezing Medium



H9 (p12 in PluriSTEM)
6 days post thaw

- High cell viability after thawing;
- Superior cell attachment
- Rapid cell proliferation; cells are ready to be passaged in 6 days.
- Undifferentiated colony morphology
- 1: 1 ratio: Typically one well of 6-well plate is frozen to 1 vial.

Figure 5. Morphology of hESC(H9) 6 days after thaw. Cell clumps from one well of H9 in a 6-well-plate were frozen down using PluriSTEM™ Freezing Medium (SCM134).

Corresponding Author: vi.chu@merckmillipore.com

www.merckmillipore.com