The advancement of feeder-independent culture systems for undifferentiated human pluripotent stem cells towards greater regulatory compliance

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Introduction

Defined and feeder-independent cell culture systems provide a platform for greater reproducibility and standardization in human pluripotent stem cell (hPSC) research. mTeSR®1 has become the most widely cited feeder independent system for the culture of undifferentiated human ES and iPSC (Table 1). As the field develops potential therapeutic applications for hPSC-derived cells, it is increasingly important that media products are manufactured to meet applicable regulatory compliance standards. Therefore, we have developed two new products for the expansion of undifferentiated hPSCs; mTeSR®1 manufactured in GMP facility (mTeSR®1-GMP) and animal protein-free TeSR™2. Here we show data to support their use for maintenance of high quality undifferentiated hPSC cultures.

Figure 1: The TeSR™ System

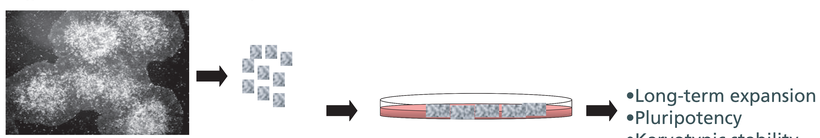


Table 1. Ten select publications using mTeSR®1 in 2009 for a variety of applications

Derivation and/or Culture of iPSC

Chan et al, 2009 Nat Biotechnol. Nov;27(11):1033-7. Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells.

Eminli et al., 2009 Nat Genet 4:968-976. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells.

Sun et al., 2009 PNAS Sep 15;106(37):15720-5. Feeder-Free derivation of induced pluripotent stem cells from adult human adipose stem cells.

Derivation of hESC

Eremeevet al., 2009 Dokl Biol Sci 426:293-295. Derivation of a novel human embryonic stem cell line under serum-free and feeder-free conditions.

Cell Source for Differentiation of hPSC

Yu et al., 2009 PLoS ONE 4(9): e7040. nAChRs mediate human embryonic stem cell-derived endothelial cells: proliferation, apoptosis, and angiogenesis.

Lu et al., 2009 Exp Hematol 37:924-936. Enhanced generation of hematopoietic cells from human hepatocarcinoma cell-stimulated human embryonic and induced pluripotent stem cells.

hESC Characterization

Zeng et al., 2009 Stem Cells Oct;27(10):2435-45. Lack of ABCG2 expression and side population properties in human pluripotent stem cells.

Kolle et al., 2009 Stem Cells Oct;27(10):2446-56. Identification of human embryonic stem cell surface markers by combined membrane-polysome translation state array analysis and immunotranscriptional profiling.

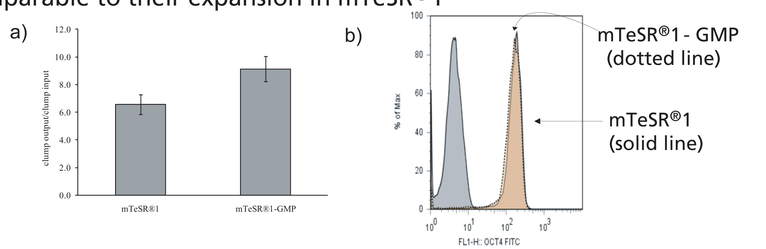
Alternate Culture Systems

Hakala et al., 2009 Tissue Eng Part A. Jul;15(7):1775-85.Comparison of biomaterials and extracellular matrices as a culture platform for multiple, independently derived human embryonic stem cell lines.

Oh et al., 2009 Stem Cell Research Mar 4. [Epub ahead of print]. Long term microcarrier suspension cultures of human embryonic stem cells.

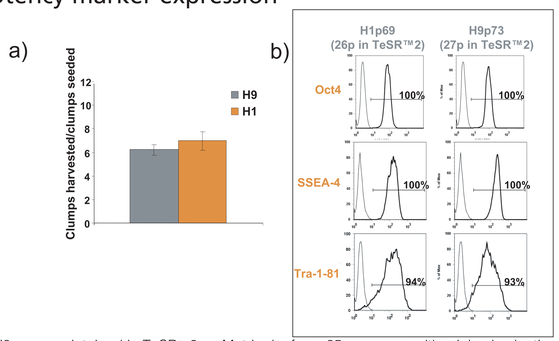
Results

Figure 2. Expansion of undifferentiated cells in mTeSR®1-GMP is comparable to their expansion in mTeSR®1



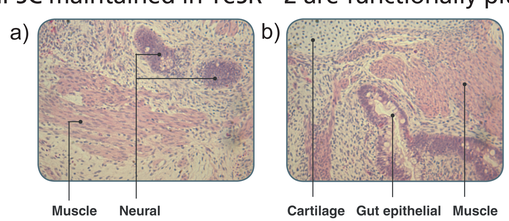
a) Cells were cultured in mTeSR®1 and mTeSR®1-GMP on Matrigel™ for 5 passages with minimal selection of differentiated colonies (<10% per passage). Expansion was measured by enumerating clumps at harvest/clumps seeded (n=2, data pooled from 4 passages each ± SEM). b) Oct3/4 expression measured by FACS was >95% at the end of 5 passages for H9 cells cultured in mTeSR®1 (orange, solid line) and mTeSR®1-GMP (pale orange, dotted line). Secondary antibody only control for mTeSR®1-GMP = grey, solid line.

Figure 3. Long-term culture of undifferentiated cells in animal protein-free TeSR™2 affords robust expansion and maintenance of pluripotency marker expression



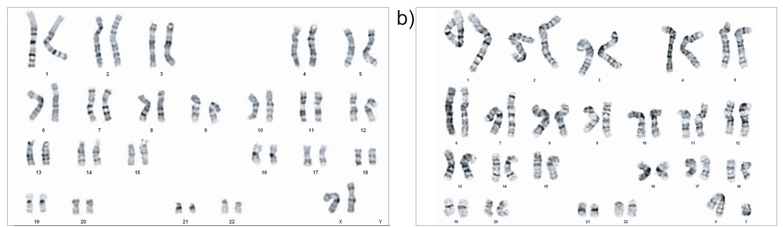
a) H1 and H9 were maintained in TeSR™2 on Matrigel™ for > 25 passages with minimal selection of differentiated colonies (<10% per passage). Average (± 1 SEM) expansion per passage was measured as described in Figure 1 (24 passages for H9; 22 passages for H1). b) FACS analysis of Oct3/4, SSEA-4 and Tra-1-81 after >25 passages in TeSR™2.

Figure 4. hPSC maintained in TeSR™2 are functionally pluripotent



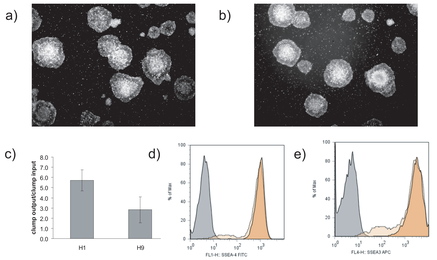
H9 cells isolated after 11 passages in TeSR™2 on Matrigel™, and then injected into mice, generated teratomas over a period of 9 weeks. a, b) Histological analysis of H&E stained sections of the resulting teratomas revealed tissues representative of all three germ layers as indicated. gut epithelia = endoderm; neural=ectoderm; cartilage, muscle=mesoderm

Figure 5. hPSC cultured in TeSR™2 maintain a normal karyotype with prolonged passaging



G band karyotype analysis of a) H9 cells (normal, XX) after 22 passages in TeSR™2 on Matrigel™ and b) H1 cells (normal, XY) after 19 passages in TeSR™2 on Matrigel™.

Figure 6. Undifferentiated growth of hPSC in TeSR™2 on a defined matrix



a) H9 and b) H1 cells were cultured in TeSR™2 on recombinant human vitronectin (R&D Systems) for 5 passages using mechanical dissociation of clumps without enzyme at each passage. Cells cultured in these conditions form tightly packed colonies and maintain their characteristic undifferentiated morphology. c) Average (± 1 SEM) expansion per passage was measured as described for Figure 1 (4 passages for each line). FACS analysis at the end of 5 passages reveals >95% SSEA4 (d) and >97% SSEA3 (e) expression by both H1 and H9 cells. Secondary antibody only control for H1 = gray, solid line, H1=pale orange, dotted line, H9=orange, solid line.

Conclusions

- mTeSR®1 manufactured in a GMP facility provides equivalent performance to mTeSR®1.
- TeSR™2 is an animal protein-free formulation that can be used with Matrigel™ or more defined matrices to maintain pluripotent and karyotypically normal hPSC.
- These two new products represent STEMCELL's ongoing commitment to provide ongoing regulatory compliant reagents to facilitate the translation of basic research to pre-clinical studies.

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