Comparison of Human Pluripotent Stem Cells Cultured on Matrigel™ and Recombinant Human Vitronectin using TeSR™-E8™ Low Protein Medium

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Introduction

TeSR-E8 has recently been introduced as a simplified, low protein version of the commercial media formulations mTeSR™1 and TeSR™2 for human pluripotent stem cell (hPSC) maintenance. TeSR-E8 was developed by STEMCELL Technologies, Inc. (STEMCELL) based on the formulation known as E8, which was reported by the Thomson lab (Chen *et al.*, 2011). E8 was developed via the pair wise removal of extraneous additives from the TeSR™ core media formulation, resulting in a low protein medium with only eight components. We tested the performance of TeSR-E8 with two different substrates, either Matrigel™ or recombinant human Vitronectin (rhVitronectin, rhVN) using multiple hPSC lines. Our results showed that we could routinely achieve high expansion and culture quality for both embryonic stem (ES) and induced pluripotent stem (iPS) cells. To enable the success of achieving long-term cultures reproducibly, it was critical that key steps in the passaging protocol were tightly regulated. For example, improper handling techniques such as incomplete dissociation or low plating density during passaging resulted in poor plating efficiencies and/or increased differentiation. We also investigated the effects of transitioning hPSCs routinely cultured on Matrigel™ matrix in TeSR-E8 medium (Matrigel™/TeSR-E8) to the alternate rhVN substrate, by assessing pluripotency and cell expansion rates obtained for the passages immediately following transition. We found that there was little change in cell expansion rates upon transitioning hPSC from Matrigel™ to rhVN, although spontaneous differentiation was observed to be slightly higher in the passages immediately following transition. As a preliminary assessment of cell function, embryoid bodies were successfully generated from cells grown on both substrates. Overall, our findings demonstrate that TeSR-E8, a low protein formulation, offers an effective alternate to TeSR™ media when used with rhVitronectin or Matrigel™ substrates.

Vitronectin as a Matrix Protein for hPSC

Vitronectin (VN) is a blood plasma glycoprotein that is present in human plasma at concentrations of 200-400 µg/ml (Preissner and Reuning, 2011). VN plays a key role in the attachment of cells to their surrounding extracellular matrix (ECM), regulation of cell migration/invasion, proliferation, and tissue remodeling. In addition, VN also serves as a modulator of cell-ECM interface with regulatory functions in the control of hemostasis, blood coagulation, pericellular proteolysis, and innate immunity. The glycoprotein sequence has a number of known binding regions (Figure 1), but cell adhesion is known to occur predominantly between the cell surface integrins and the RGD domain of VN.

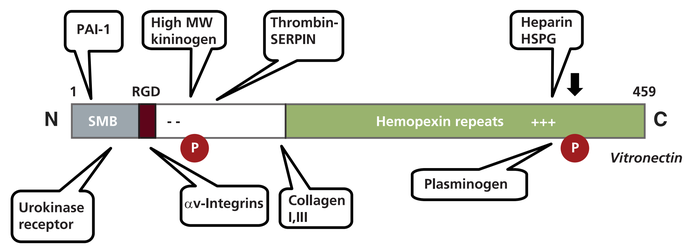
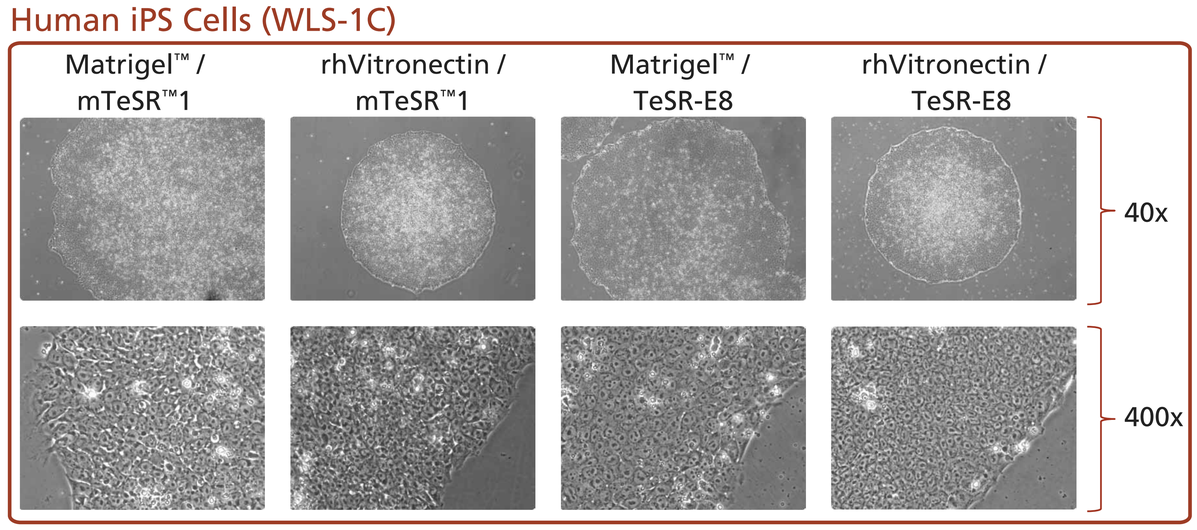


Figure 1. Schematic representation of the human vitronectin protein sequence and associated binding regions (adapted from Preissner and Reuning, 2011).

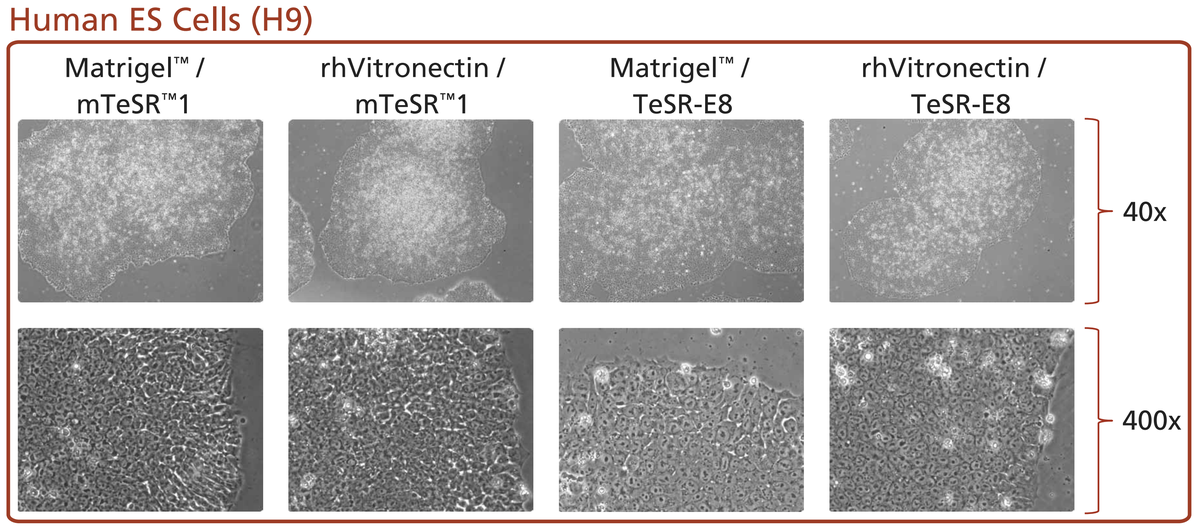
Cell Morphology

hPSCs cultured on rhVN retain the prominent nucleoli and high nuclear to cytoplasm ratio that are characteristic of this cell type. Dense, bright centers are prominent when cells are ready to passage due to multilayering of cells at the center of the colony. Cells have similar morphology both on Matrigel™ and rhVN-coated surfaces in either mTeSR™1 or TeSR-E8 medium.

Human iPS Cells (WLS-1C)



Human ES Cells (H9)



Methods & Materials

Cultureware surfaces were coated with a 10 µg/mL rhVN solution. hPSCs that were cultured on Matrigel™ matrix with mTeSR™1 medium (Matrigel™/mTeSR™1) or on Matrigel™/TeSR-E8 (see Table 1 for a comparison of media components) maintenance cultures were seeded into rhVN-coated plates to initiate the experiments. Cells were passaged every 5-7 days for up to 20 passages using an enzyme-free passaging method. Briefly, media was aspirated from wells to be passaged and cells were exposed to Gentle Cell Dissociation Reagent (STEMCELL) for 6-10 minutes, at which time cells began to exhibit loosened packing within the colony (Figure 2). The buffer was then aspirated before adding an appropriate volume of complete medium to the well. Clumps were harvested by scraping attached colonies with a glass pipette and breaking up to desired clump size via repeated pipetting as necessary. The dissociated clumps were then diluted according to the desired split ratio and replated into a new rhVN-coated plate in the appropriate medium. At each passage, cells were characterized by assessing plating efficiency (# clumps seeded/# clumps attached at 48 h), colony morphology, and clump expansion (#clumps harvested/# clumps seeded). It was critical that key steps in the passaging protocol be tightly regulated. Cultures performed better when maintained in TeSR-E8 media at approximately 3x higher density than those cultured in mTeSR™1 (Figure 3). Following these methods, we were able to maintain high quality hPSC for multiple passages on rhVN with mTeSR™1 or TeSR-E8 without the use of dissociation enzymes.

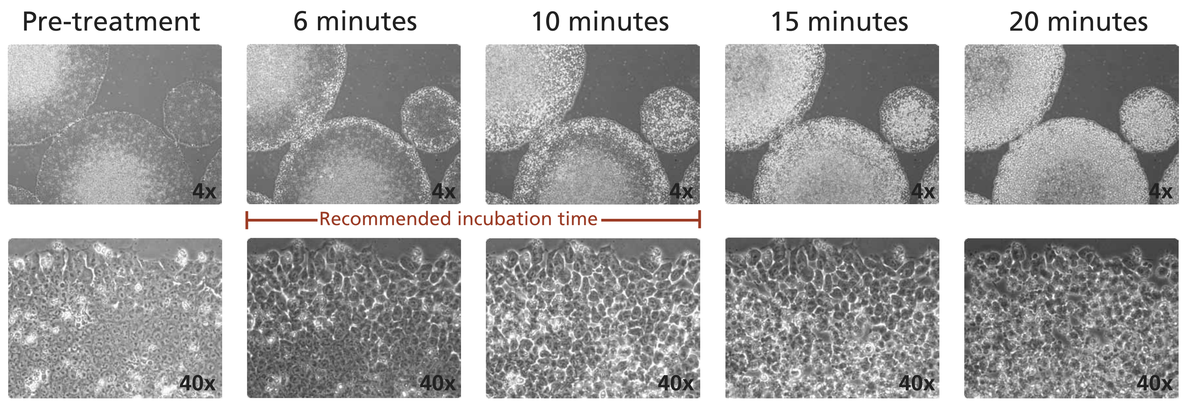


Figure 2. Effect of Gentle Cell Dissociation Reagent on colony detachment. Colonies dissociate to appropriate amounts after approximately 6-10 minutes. Longer exposure times may result in the generation of an undesired level of single cells upon detachment.

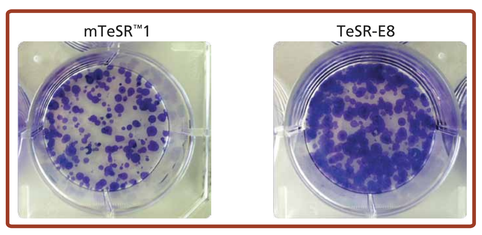


Figure 3. An example of Giemsa staining of mTeSR™1 and TeSR-E8 hPSC cultures used to demonstrate the appropriate colony density when cultures are ready for passaging. Note that the TeSR-E8 cultures are seeded at approximately 3x higher colony density than mTeSR™1 cultures.

Component	mTeSR™1	E8
DF12	√	√
$NaHCO_3$	√	√
L-Ascorbic Acid	√	√
Selenium	√	√
Transferrin	√	√
Insulin	√	√
FGF2	√	√
TGF-β	√	√
BSA	√	x
Glutathione	√	x
Trace Elements	√	x
BME	√	x
Pipecolic Acid	√	x
GABA	√	x
Lithium Chloride	√	x
Defined Lipids	√	x

Table 1. Comparison of components in mTeSR™1 and E8 medium.

Characterization

Cells cultured using rhVN/TeSR-E8 were characterized using standard assays such as fluorescence-activated cell sorting (FACS) for pluripotency markers and karyotype analysis. This analysis shows that cells grown on rhVN are of high quality as determined by their pluripotent characteristics.

Table 2.

Cell Type	Cell Line	OCT4	SSEA3
hESC	H1	97.0%	94.4%
	H9	98.7%	99.5%
hiPSC	WLS-1C	99.2%	98.6%
	WLS-4D1	97.3%	99.2%
	A13700	98.3%	99.9%

Figure 5

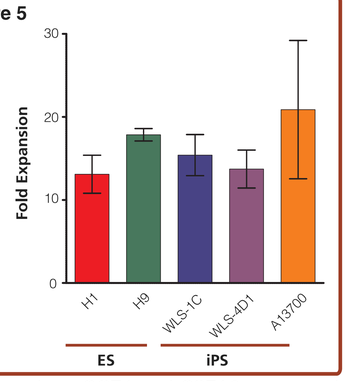


Figure 6

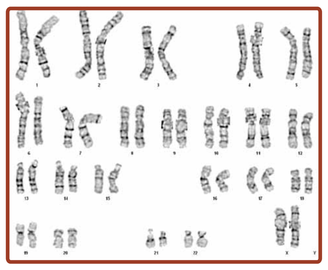


Table 2. Summary of FACS results for pluripotency markers OCT4 and SSEA3 expressed by cells at the end of five passages with rhVN/TeSR-E8. **Figure 5.** Graph showing the average expansion for cell lines grown with rhVN/TeSR-E8 over 5 passages. **Figure 6.** Representative karyotype for the H9 cell line after 10 passages in rhVN/TeSR-E8 shows no chromosomal abnormalities.

Embryoid Body Formation of hPSC

In separate experiments, it was demonstrated that rhVN/TeSR-E8 cultured cells retain the ability to form embryoid bodies, a common starting point for differentiation protocols, in a size-controlled system. Single cells were generated from H1 hES maintenance cultures using standard techniques, and the cells were then placed in an AggreWell™400 plate containing AggreWell™ medium with 10 µM Y-27632 (STEMCELL). After 24 hours, the embryoid bodies were transferred to an ultra low adherence (ULA) plate for inspection.

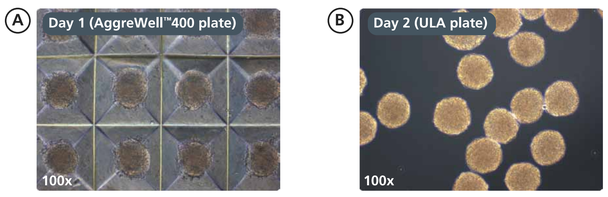


Figure 7. Image of embryoid bodies generated from H1 hES cells cultured using rhVN/TeSR-E8 within an AggreWell™400 plate (A) and in suspension after harvesting (B).

Conclusions

rhVitronectin is a completely defined, recombinant human protein that can be used to support the long-term maintenance of hPSC while retaining high pluripotency and functionality of these cells. When used with mTeSR™1 or TeSR-E8 maintenance media, rhVitronectin provides a completely defined system in which hPSCs behave functionally equivalent to those maintained on the undefined Matrigel™ matrix. This system allows complete control over the culture environment, resulting in more consistent cell populations and more reproducible results in downstream applications.