Culture of human ES and iPS cells using TeSR™-E8™: A simplified and low protein maintenance medium compatible with either BD Matrigel™ or Vitronectin XF™

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Introduction

The media, protocols and culture systems used for human embryonic stem (ES) and induced pluripotent stem (iPS) cells under feeder-free conditions have undergone steady improvements since their widespread use was adopted during the last decade. A key contributor to this progress has been the defined, reproducible and feeder-free maintenance medium and culture conditions provided by commercially available mTeSR™1 (STEMCELL Technologies), which was developed based on publications from James Thomson's laboratory [Ludwig et al., 2006]. Further developments by the Thomson laboratory have demonstrated that it is possible to remove some extraneous additives and most of the protein content from the base mTeSR™1 formulation, resulting in a simplified medium known as E8 [Chen et al., 2011]. A commercially manufactured version of the E8 formulation, TeSR™-E8™ (STEMCELL Technologies), is now available as an alternative maintenance medium for human ES and iPS cell culture. The aim of this study was to test the robustness of new TeSR™-E8™ by assessing cell morphology, cell expansion and pluripotent marker expression in two ES (H1, H9) and three iPS (WLS-1C, WLS-4D1, A13700) cell lines cultured in TeSR™-E8™ in the presence of two different culture matrices: Matrigel™ and Vitronectin XF™ (Developed and manufactured by Primorigen Biosciences).

Defined Culture with TeSR™-E8™ & Vitronectin XF™

Vitronectin (VN) is a blood plasma glycoprotein that is present in the plasma at concentrations of 200 - 400 µg/ml [Preissner and Reuning, 2011]. VN plays a key role in 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 the cell-ECM interface with regulatory functions in the control of hemostasis, blood coagulation, pericellular proteolysis and innate immunity. Vitronectin 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. Vitronectin has previously been shown to support human pluripotent stem cell (hPSC) culture with defined media such as mTeSR™1 [Braam and Mummery, 2008], but its widespread use has been hindered due to the unavailability of a cost-effective and robust material that is qualified for this application. Vitronectin XF™ is a fully humanized, recombinant VN protein that is qualified for use with simplified TeSR™-E8™ medium (Table 1) to provide a fully defined system for human ES and iPS cell culture.

Component	mTeSR™1	TeSR™-E8™
DMEM/F12	●	●
$NaHCO_3$	●	●
L-Ascorbic Acid	●	●
Selenium	●	●
Transferrin	●	●
Insulin	●	●
FGF2	●	●
TGF-β	●	●
BSA	●	
Glutathione	●	
Trace Elements	●	
BME	●	
Pipecolic Acid	●	
GABA	●	
Lithium Chloride	●	
Defined Lipids	●	
Total Protein (g/L)	~13	~0.03

Table 1. Comparison of components in mTeSR™1 and TeSR™-E8™. Note that total protein is ~433 fold lower in TeSR™-E8™ compared to mTeSR™1.

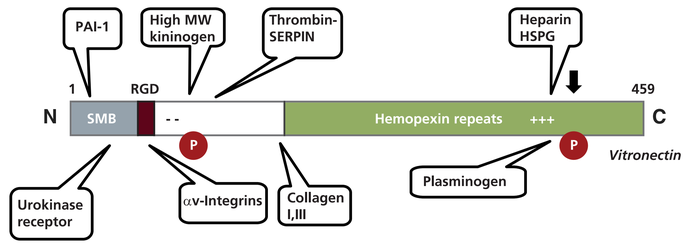

Figure 1. Schematic representation of the human vitronectin protein sequence and associated binding regions (adapted from Preissner and Reuning, 2011).

Materials and Methods

Culture Matrices: Cultureware was coated with either a 10 µg/mL solution of Vitronectin XF™ (STEMCELL Technologies), or Matrigel™ (BD Biosciences) according to the supplier recommendations. hPSCs that were cultured on Matrigel™ matrix with mTeSR™1 medium were seeded onto the coated plates to initiate the experiments.

Passaging Cells: Cells were passaged every 5 - 7 days for up to 20 passages using an enzyme-free passaging method. Briefly, the medium was aspirated from wells to be passaged and cells were exposed to Gentle Cell Dissociation Reagent (STEMCELL Technologies) for either 6 - 8 minutes (Matrigel™) or 10 - 12 minutes (Vitronectin XF™), at which time cells began to exhibit loosened packing within the colony (see Figure 2). The buffer solution was then aspirated before adding TeSR™-E8™ medium to the well. Cell aggregates were harvested by scraping attached colonies with a cell scraper and breaking up to the desired aggregate size via repeated pipetting.

Cell Plating: The dissociated aggregates were then diluted according to the desired split ratio and replated into a pre-coated plate in the appropriate medium.

Culture Assessment: At each passage, cells were characterized by assessing plating efficiency (#aggregates seeded / #aggregates attached at 48 h), colony morphology and fold expansion (#aggregates harvested / #aggregates seeded). Following these methods, we were able to maintain high quality hPSCs with both Matrigel™ and Vitronectin XF™ for multiple passages in TeSR™-E8™.

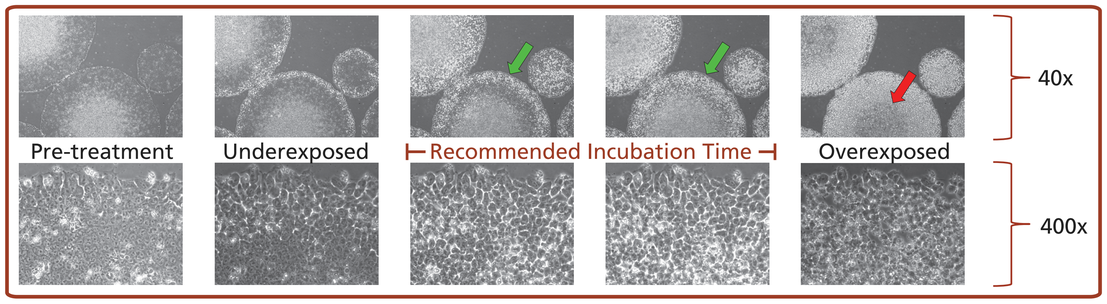

Figure 2. Effect of Gentle Cell Dissociation Reagent on colony detachment. In this example, colonies grown on Vitronectin XF™ dissociate to appropriate amounts after approximately 10 - 12 minutes. At the optimal time of dissociation, gaps should be visible between cells at the outside of the colonies (green arrows), but should not have penetrated fully through the center of the colony (red arrow). Note that exposure time should be optimized for individual culture conditions to avoid the generation of an undesired level of single cells upon detachment.

Cell Morphology

Human ES and iPS cells cultured in TeSR™-E8™ retain the prominent nucleoli and high nuclear to cytoplasm ratio characteristic of these cell types. Colonies grown in TeSR™-E8™ have a more condensed and round morphology when grown on Vitronectin XF™ matrix, compared to colonies grown on Matrigel™, which are more diffuse and irregularly shaped.

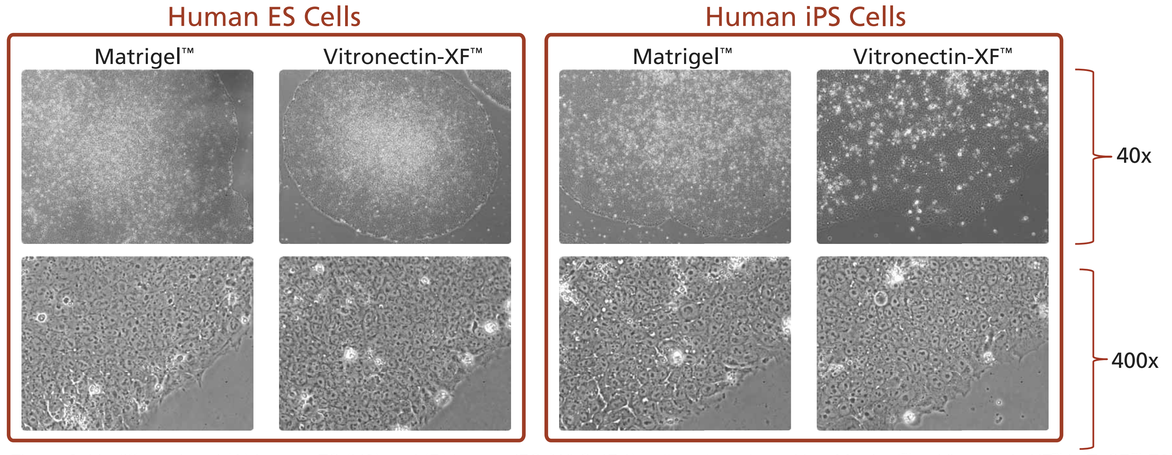

Figure 3. Undifferentiated (A) human ES (H9) and (B) human iPS (WLS-4D1) cells cultured on either Matrigel™ or Vitronectin XF™ in TeSR™-E8™. Densely packed cells and multilayering are prominent when cells are ready to passage.Images are representative of cultures at the optimal time of passaging.

Characterization of hPSCs cultured in TeSR™-E8™

Cells were passaged in TeSR™-E8™ medium for 10 - 20 passages and characterized using standard assays such as flow cytometry for pluripotency markers (SSEA3, Oct4 and Tra-1-81), expansion rate measurement, karyotype analysis and qualitative morphological assessment. Cells grown in TeSR™-E8™ were found to have comparable performance on both Matrigel™ and Vitronectin XF™ matrices, and retained the accepted characteristics of highly pluripotent, quality hPSC cultures (Figures 4 - 6).

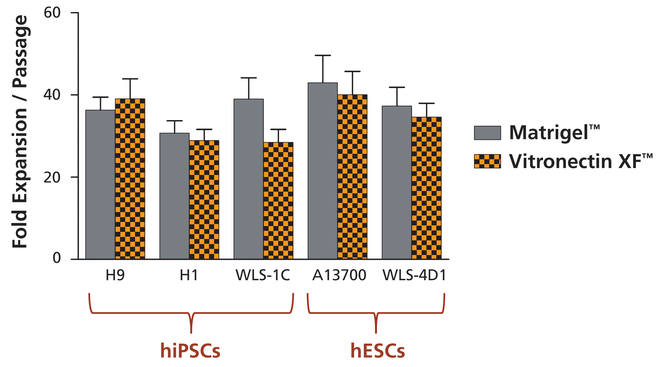

Figure 4. Graph shows the average fold expansion per passage ± SEM obtained for human ES and iPS cells cultured in TeSR™-E8™ with either Matrigel™ (solid bars) or Vitronectin XF™ (checkered bars) over 10 passages. Note that this data is representative of cultures passaged after 6 - 7 days in culture; lower expansion should be expected if using shorter culture times.

Figure 5. Representative karyotype analyses for H9 ES cells after 10 passages in TeSR™-E8™ with either Matrigel™ or Vitronectin XF™ show no genetic abnormalities.

% Oct 4 Expression After 10 Passages with TeSR™-E8™

Cell Line	Vitronectin XF™	BD Matrigel™
H9	95.7	98.0
H1	98.2	98.0
A13700	97.3	99.0
WLS-1C	99.9	99.9
WLS-4D1	99.7	99.9

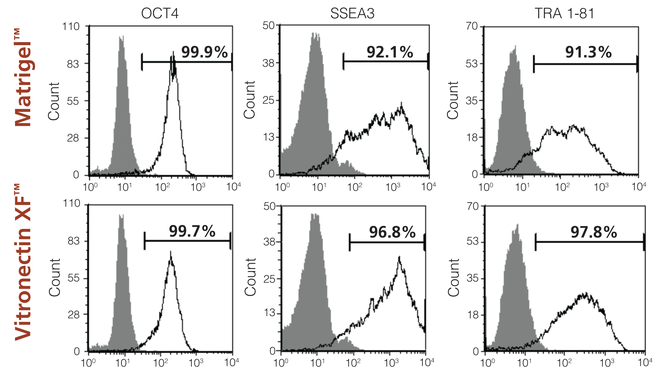

Figure 6. Pluripotency marker expression was assessed by flow cytometry after culturing for 10 passages in TeSR™-E8™ medium with either Matrigel™ or Vitronectin XF™. (Left) Expression of pluripotency marker Oct4. (Right) Histogram of pluripotency markers (Oct4, SSEA3 and Tra-1-81) for WLS-4D1 human iPS cells after 10 passages in TeSR™-E8™ (black = sample, gray = secondary antibody only).

Summary

TeSR™-E8™ is a simplified medium for the maintenance of human ES and iPS cells, and can be used in combination with either Matrigel™ or Vitronectin XF™ as the culture matrix. Performance was comparable between these two matrices and cells were found to retain the characteristics of highly pluripotent stem cell cultures. Our results indicate that TeSR™-E8™ and Vitronectin XF™ provide a robust and fully-defined culture system that maintains high quality cell populations for downstream applications.

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