NutriStem® hPSC XF Medium Supports Long-term Culture of Human Pluripotent Stem Cells on Corning® Matrigel® hESC-qualified Matrix

Application Note

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Introduction

Human pluripotent stem cells (hPSCs) have numerous applications in cell therapy and regenerative medicine[1]. Human PSCs including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have the potential for indefinite self-renewal and ability to differentiate into cells of all three germ layers[2]. Human PSCs have been traditionally co-cultured with irradiated mouse embryonic fibroblast (MEF) cells in the presence of serum-containing medium. Co-culture with MEFs is labor-intensive, and introduces variability. In an effort to create a better defined culture system, researchers have cultured hPSCs on Corning Matrigel matrix (a mouse tumor-derived basement membrane extract) in the presence of MEF-conditioned medium containing serum-replacement supplement[3]. Although this system eliminated the variability associated with serum, it still contains unknown components secreted by the MEFs into the medium. Thus, there is a need to develop better defined serum-free, feeder-free culture environments for long-term hPSC culture.

NutriStem hPSC XF medium has been used for long-term culture of hPSCs on recombinant Laminin-521 coated surface[4,5], and Corning PureCoat™ rLaminin-521 cultureware[6]. In this study, NutriStem hPSC XF medium, a defined, serum-free and xeno-free medium, was used to support the long-term culture of hiPSCs on Corning Matrigel hESC-qualified matrix. Throughout the ten passage experiment, the cells remained undifferentiated, retained their pluripotency and possessed a normal karyotype.

Materials and Methods

Human induced pluripotent stem cell (hiPSC) passaging on Corning Matrigel hESC-qualified matrix

A human episomal iPSC line (Thermo Fisher) was used for this study and was routinely maintained on Matrigel hESC-qualified matrix (Corning) as previously described.[6]

Long-term hiPSC culture in NutriStem hPSC XF medium

NutriStem hPSC XF medium (manufactured by Biological Industries) was thawed and used according to the manufacturer's instructions.

Note: NutriStem hPSC XF medium can now be purchased from Corning: Cat. Nos. 40-05-100-1A (500 mL) and 40-05-100-1B (100 mL).

Human iPSCs were seeded on Matrigel matrix coated plates in NutriStem hPSC XF medium. To passage the cells as clumps on Matrigel matrix, the cells were washed with DPBS (2 mL/well; without calcium and magnesium), cell dissociation buffer (1 mL/well; enzyme-free PBS, Thermo Fisher) was added and cells were incubated for 3 to 5 minutes at room temperature. Separation of the cells within the colonies was observed under the microscope. The cell dissociation buffer was carefully aspirated from the wells without disrupting the adhered cells. Fresh NutriStem hPSC XF medium was forcefully added to each well to dislodge the cells as small clusters. The small cell clumps/clusters were seeded onto a freshly-coated Matrigel matrix plate in fresh NutriStem hPSC XF medium (3 mL/well) and incubated at 37°C and 5% CO_2 in a humidified incubator. A complete medium change was performed 48 hours. after cell seeding and cells were fed daily until they were ready to be passaged. Cells were passaged every 3 to 5 days when the colonies were large, beginning to merge, and had centers that were dense and phase-bright compared to their edges. Colonies were passaged at a split ratio of 1:14 to 1:21. Cell morphology was observed daily using an EVOS® ci Cell Imaging System (AMG).

Undifferentiated marker expression using flow cytometry analysis

Cells were dissociated from Matrigel matrix-coated plates with Accutase® and samples were prepared for FACS according to the manufacturer's instructions for the BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit (Becton Dickinson). The cells were analyzed with a FACSCalibur™ flow cytometer (Becton Dickinson) and data were analyzed with the CellQuest™ Pro software (Becton Dickinson).

Tri-lineage differentiation

Pluripotency of hiPSCs was investigated using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) according to the manufacturer's instructions. Plates (24-well format) were coated with Matrigel matrix, hiPSCs were plated and differentiated into the three lineages. The three germ layers were stained with antibodies for Otx2 (ectoderm marker), brachyury (mesoderm marker), and SOX17 (endoderm marker). The cells were counterstained with the corresponding secondary antibody (Donkey anti-goat IgG NorthernLights™ NL557-conjugated antibody, R&D Systems) and nuclei were labeled with Hoechst 33342 (Thermo Fisher). The stained differentiated cells were imaged using a fluorescence microscope (Olympus IX70).

Karyotype analysis

Karyotyping by G-banding analysis was performed by Cell Line Genetics® (WI).

Results and Discussion

Human episomal iPSCs were cultured in NutriStem® hPSC XF medium for ten passages on Corning® Matrigel® matrix coated plates. During this experiment, the hiPSCs exhibited the typical undifferentiated hPSC morphology as characterized by the presence of small cells and high nucleus to cytoplasm ratio (Figure 1).

Cells remained undifferentiated during this long-term culture study as demonstrated by the marker expression profile from flow cytometry analysis (Figure 2). After ten passages, hiPSCs expressed undifferentiated hPSC markers Oct-3/4 (>94.5%) and SSEA-4 (>99%) while the differentiation marker SSEA-1 (0.08%) was not detected.

After ten passages, hiPSCs were subjected to directed differentiation to endoderm, mesoderm, and ectoderm and the cells were characterized using germ layer-specific markers: Otx2 for ectoderm, brachyury for mesoderm, and SOX17 for endoderm (Figure 3). Human iPSCs cultured in NutriStem hPSC medium on Matrigel matrix were able to differentiate into the three germ layers demonstrating that NutriStem hPSC medium together with Matrigel matrix supported the expansion of hPSCs while maintaining their pluripotency.

After ten passages, hiPSCs exhibited a normal karyotype and genetic abnormalities were not detected (Figure 4).

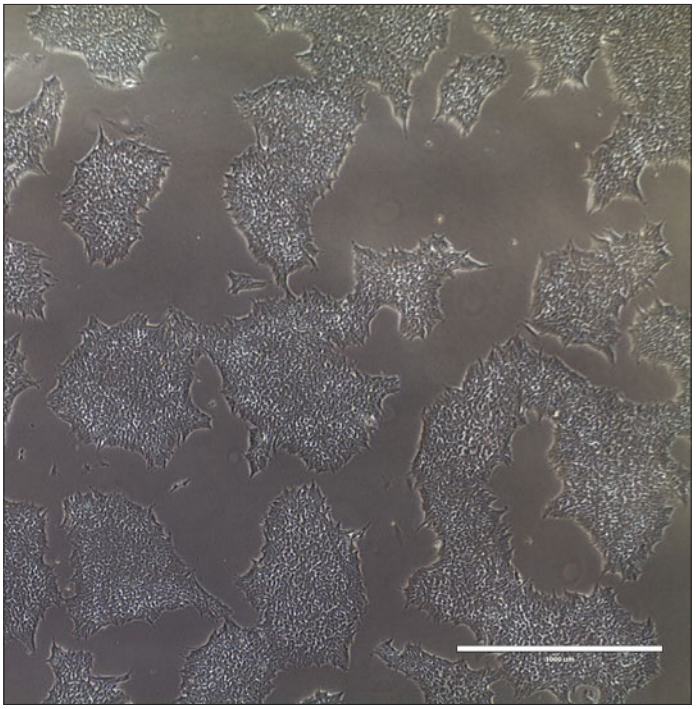

Figure 1. Representative image of hiPSCs cultured in NutriStem hPSC XF medium on Corning Matrigel matrix from day 3 of passage 3. Scale bar = 1,000 µm.

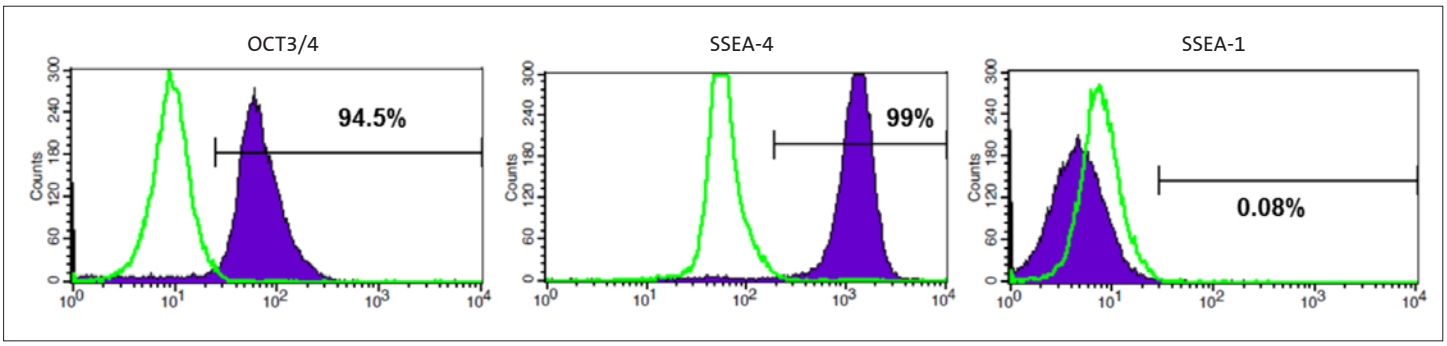

Figure 2. Histograms from flow cytometry showing the expression of Oct-3/4, SSEA-4, and SSEA-1 after ten passages in NutriStem hPSC medium on Corning Matrigel matrix.

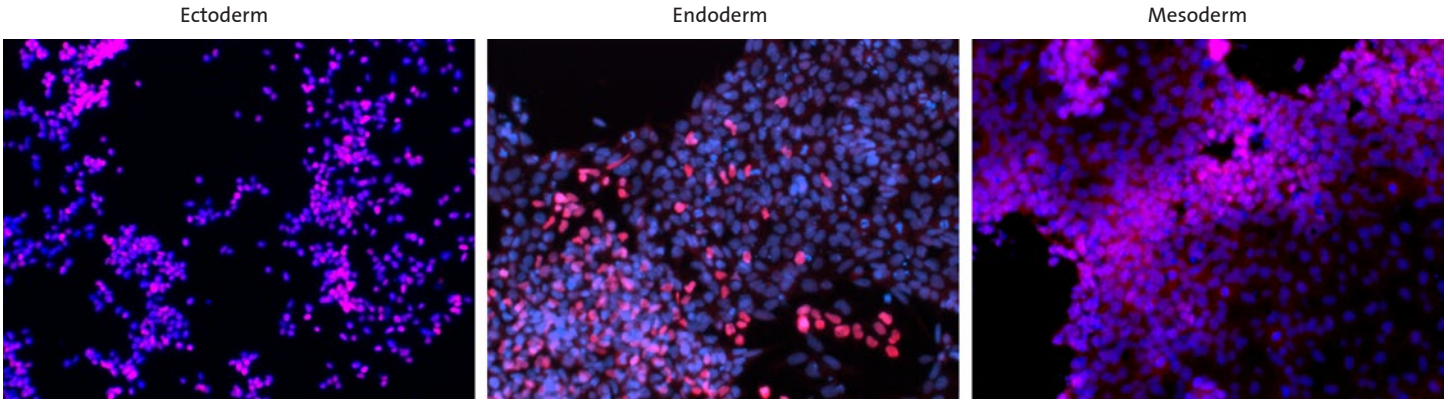

Figure 3. Representative fluorescence micrographs of hiPSCs differentiated into three germ layers and immunostained with corresponding antibodies (red): ectoderm (Otx2), endoderm (SOX17), and mesoderm (brachyury). Cell nuclei were labeled using Hoechst 33342 (blue).

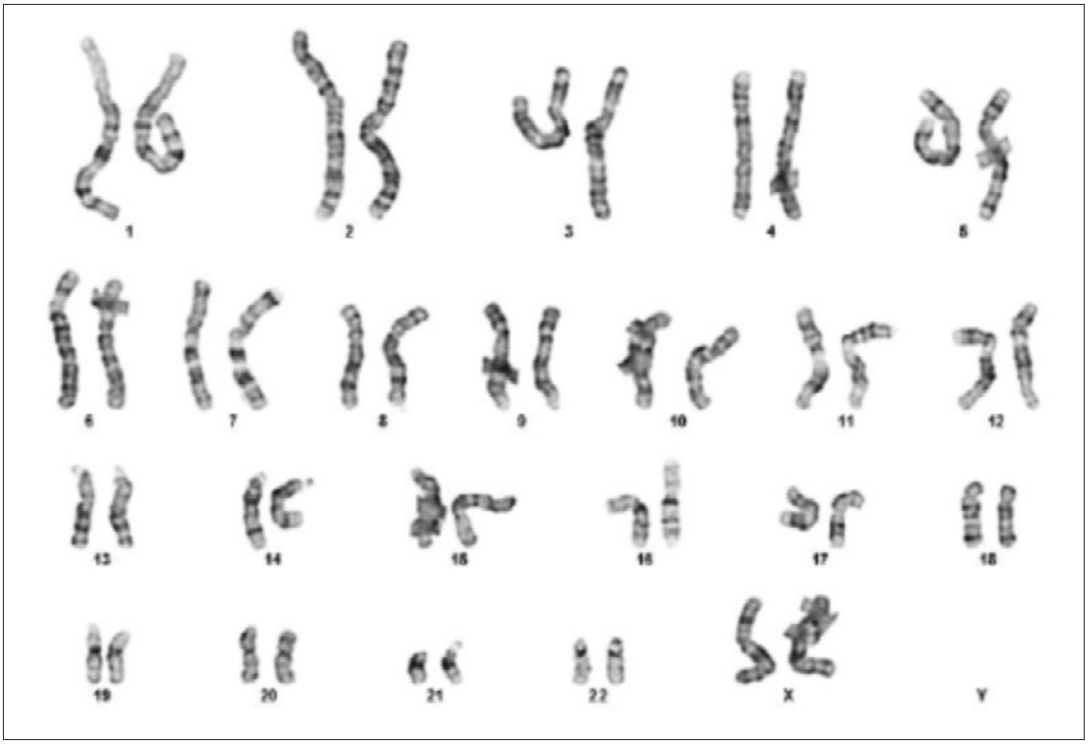

Figure 4. Karyotype of hiPSCs after ten passages in NutriStem hPSC medium on Corning Matrigel matrix.

Conclusions

NutriStem® hPSC XF medium is a serum-free and xeno-free medium for the culture of hPSCs. In this study, NutriStem hPSC XF medium supported the long-term culture of hiPSCs as clumps on Corning® Matrigel® matrix. During this ten passage study, the cells had typical hPSC morphology, remained undifferentiated, retained their ability to differentiate into all three germ layers, and possessed a normal karyotype.

References

1. Carpenter, MK and Rao, MS, Concise review: making and using clinically compliant pluripotent stem cell lines, Stem Cells Translational Medicine (2015), 4(4):381-388.
2. Villa-Diaz, LG, et al., Concise Review: The evolution of human pluripotent stem cell culture: from feeder cells to synthetic coatings, Stem Cells, (2013), 31(1):1-7.
3. Xu C, et al., Feeder-free growth of undifferentiated human embryonic stem cells, Nature Biotechnology (2001), 19(10):971-974.
4. Rodin S, et al., Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment, Nature Communications (2014), 5, Article number: 3195 doi:10.1038/ncomms4195.
5. Rodin S, et al., Monolayer culturing and cloning of human pluripotent stem cells on laminin-521–based matrices under xeno-free and chemically defined conditions, Nature Protocols (2014), 9:2354-2368.
6. Nandivada H, et al., Corning PureCoat rLaminin-521 cultureware: A ready-to-use, animal-free vessel platform for the culture and single cell passaging of human pluripotent stem cells, Corning Application note: CLS-DL-AN-373, (2016).

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