Feeder-Free Expansion of Human Induced Pluripotent Stem Cells (hiPSC) on Corning® Synthemax® II-SC Substrate Coated Surface in Defined Medium

SnAPPShots

A brief technical report from the Corning Development Group

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Introduction

Current methods to culture pluripotent stem cells involve complex animal-derived products as attachment surfaces such as mouse feeder layers, Matrigel,™ murine laminin, or various human-derived biological substances. Most of these materials are costly, of limited scalability and applicability, have batch to batch variability, and are a potential source of adventitious agents. To overcome these challenges, Corning commercialized the Corning Synthemax Surface, a fully synthetic, xeno-free, ready-to-use biomimetic surface for the culture of pluripotent stem cells (CLS-AN-151 REV4, CLS-AN-179).

Corning has expanded this product line to include the Corning Synthemax II-SC Substrate, a peptide-copolymer powder that readily dissolves in water, for use as a cell adhesion promoting surface coating for various stem cell lines. Corning Synthemax II-SC Substrate can be coated onto any culture vessel format providing additional flexibility to end users.

This report describes the use of Corning Synthemax II-SC Substrate, coated on Corning CellBIND® Surface 6 well plates, for the multi-passage expansion of human iPSC in defined medium. Cells maintained consistent doubling time, expression of pluripotency markers, and normal karyotype when cultured on Corning Synthemax II-SC Substrate for seven sequential passages in mTeSR®1 medium.

Materials

Cells

- Gibco® Episomal Human iPSC (Invitrogen, Cat. No. A13700)
- CF-1 MEF (Global Stem, Cat. No. S1520-100)

Cell Culture Medium

- mTeSR®1 (Stem Cell Technologies, Cat. No. 05850)
- Mouse Embryonic Fibroblast (MEF) Conditioned Medium (R&D Systems, Cat. No. AR005)

Cell Culture Surfaces

- Corning Synthemax-R Surface 6 Well Plates (Corning, Cat No. 3979)
- GFR-Matrigel (BD Biosciences, Cat. No. 356231)
- Geltrex® (Life Technologies, Cat. No. A1413202)
- Tissue Culture Treated (TCT) 6 Well Plates (Corning, Cat. No. 3516)
- Corning Synthemax II-SC Substrate (Corning, Cat. No. 3535XX1)
- Corning CellBIND Surface 6 Well Clear Multiple Well Plates, flat bottom, with lid, sterile (Corning, Cat. No. 3335)

The source for materials listed below is provided as an example; equivalent reagents from preferred vendors may be used.

- Cell Culture Water (Lonza, Cat. No. 17-724F)
- Dulbecco's Phosphate Buffered Saline (D-PBS) without Ca^{2+} or Mg^{2+} (Invitrogen™ (Cat. No. 14190-144)
- KnockOut™ DMEM (KO-DMEM) (Invitrogen Cat. No. 10829-018)

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- Dispase, 1 mg/mL (StemCell Technologies, Cat. No. 07923)
- Ethylenediaminetetraacetic Acid (EDTA) Solution, 0.02% in DPBS (0.5 mM), sterile-filtered (Sigma, Cat. No. E8008)
- Fetal Bovine Serum (FBS) Certified One Shot (Gibco, Cat. No. 0325)
- Plastic Scraper, Small (Corning Cat. No. 3010)
- 0.22 µm Filter Units (Corning Cat. No. 430767)
- 50 mL Centrifuge Tubes (Corning Cat. No. 430290)
- CryoStor™ CS-10 Defined, Animal Protein-Free Cryopreservation Medium (StemCell Technologies, Cat. No. 07930)
- 2 mL Cryogenic Vials (Corning Cat. No. 430661)
- Cryo 1°C Freezing Container, "Mr. Frosty" (Nalgene, Cat. No. 5100-0001)
- Aspirating Pipettes (Corning Cat. No. 9099)
- Storage Bottle (Corning Cat. No. 431175, 430281)
- Stripette® Serological Pipets (Corning Cat. Nos. 4487, 4488, 4489)

Methods

hiPSC Recovery from Cryopreservation and Adaptation to Corning® Synthemax®-R Surface

hiPS cells were thawed onto mouse embryonic feeder (MEF) cells in MEF-conditioned medium (MEF-CM), according to manufacturer's recommendations. At passage 2, cells were seeded onto Geltrex in MEF-CM. Culture medium was changed to mTeSR1 at passage 3, and cells were maintained under these conditions until stable doubling time was observed. For cell scale up and banking, the culture surface was changed to Corning Synthemax Surface, and cells were maintained on Corning Synthemax Surface in mTeSR1 prior to evaluation of Corning Synthemax II-SC Substrate.

Note: The direct transition of iPSC from MEF or Matrigel™ coated surface to Corning Synthemax II-SC Substrate coated surface might require some adaptation period and will need to be optimized for each iPSC line.

Surface Coating Protocols

To coat culture vessels with Corning Synthemax II-SC Substrate, a working solution was dispensed to Corning CellBIND® Surface 6 well plates at a concentration of 5 µg/cm^2 according to the provided protocol (CLS-AN-204). Excess solution was removed from the wells after incubation, and plates were seeded with cells. Extra plates were allowed to dry with the lid on at room temperature for about 30 minutes and were stored dry at 4°C for the duration of the multi-passage study. To coat culture vessels with Matrigel, a 1:30 dilution of GFR-Matrigel in KO-DMEM was dispensed to TCT 6 well plates per manufacturer's recommendation. Plates were incubated for 1 hour at room temperature, followed by storage at 4°C overnight. Coated plates were removed from storage prior to seeding and allowed to equilibrate to room temperature. Excess plates were stored at 4°C for up to 1 week.

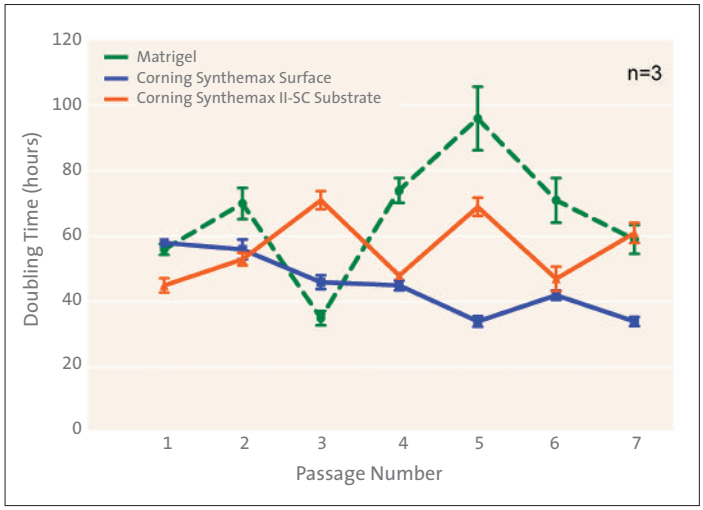


Figure 1. hiPSC doubling time on Corning Synthemax II-SC Substrate coated surface over 7 sequential passages in defined medium. Doubling time is shown for hiPSC cultured on Matrigel coated surface, Corning Synthemax Surface, and Corning Synthemax II-SC Substrate coated surface for 7 sequential passages in mTeSR1 medium. Each data point represents the average of 3 wells.

General hiPSC Passaging Protocol

hiPSC were seeded onto Matrigel coated surface, Corning Synthemax Surface, and Corning Synthemax II-SC Substrate coated surface in mTeSR1 medium. Cultures were passaged every 4 to 5 days, as cells reached 90% confluence, by incubation with 1 mg/mL dispase, followed by a brief dPBS wash and gentle scraping with a small plastic scraper. Seeding density was about 60,000 cells/cm^2. Cells received fresh medium every day, except for the day after passaging.

hiPSC Characterization

Cultures were routinely examined microscopically for cell and colony morphology. Cell viability and number were assessed at the end of each passage by harvesting three wells of each 6 well plate with 0.02% EDTA solution, followed by cell counting with an automated cell number/viability analyzer, Vi-Cell™ (Beckman Coulter). Expression of hiPSC markers Oct4 and SSEA4 were assessed at the end of each passage via immunofluorescent staining and flow cytometry analysis (FACSCalibur, BD Biosciences, CellQuest Pro analysis software). To monitor genomic integrity, cell samples for all conditions were submitted for karyotype analysis by G-banding (WiCell Research Institute Cytogenetics Lab) at passage 6.

Results/Discussion

The results demonstrate efficient attachment and expansion of hiPSC on Corning Synthemax II-SC Substrate coated surface over seven sequential passages. Cell doubling time (Fig. 1), morphology (Fig. 2), and expression of pluripotency markers (Fig. 3) were comparable for cells cultured on Matrigel coated surface, Corning Synthemax Surface, and Corning Synthemax II-SC Substrate coated surface. Normal karyotype at passage 6 was confirmed by G-banding analysis (Fig. 4).

In Fig. 1, cell doubling time is shown for seven sequential cell passages on Matrigel™ coated surface, Corning® Synthemax® Surface, and Synthemax II-SC Substrate coated surface. Comparable doubling time (average of 45 to 65 hours), viability (~80%, data not shown), and fold expansion (average of 4- to 5-fold per passage, data not shown) were observed for all surfaces. Average cell doubling times of cells cultured on Corning Synthemax Surface (45 ±10 hours) and Corning Synthemax II-SC Substrate coated surface (56 ±11 hours) were more consistent than that of cells cultured on Matrigel coated surface (66 ±19 hours).

Representative morphology pictures for hiPSC cultured on Matrigel coated surface, Corning Synthemax Surface, and Corning Synthemax II-SC Substrate coated surface are shown in Fig. 2. Cells on all surfaces exhibited typical hiPSC morphology with tight colonies, high nuclear-to-cytoplasm ratio, well-defined colony borders, and a low stromal cell

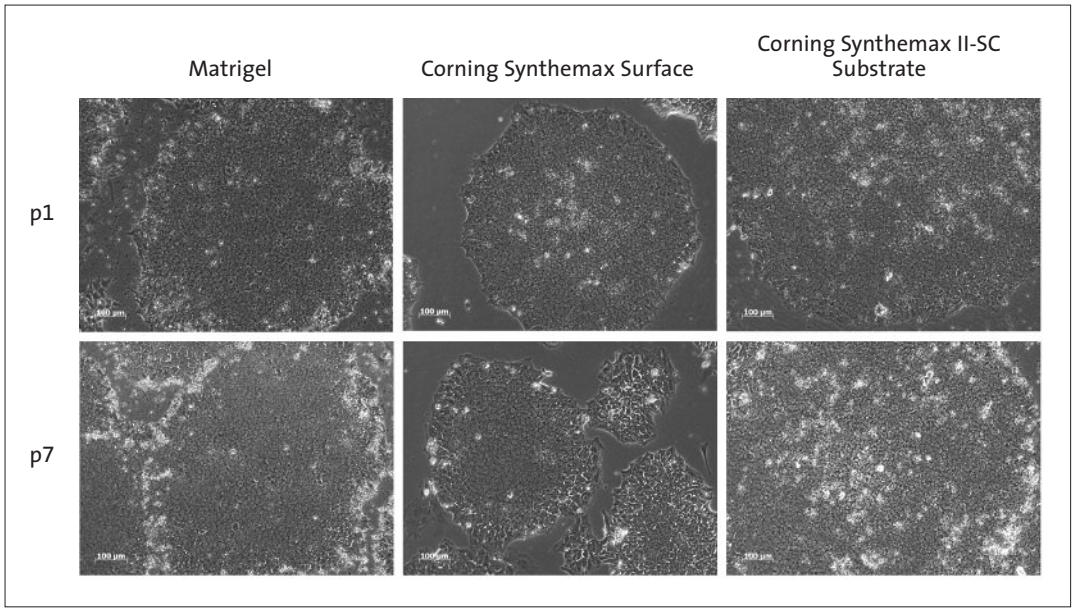
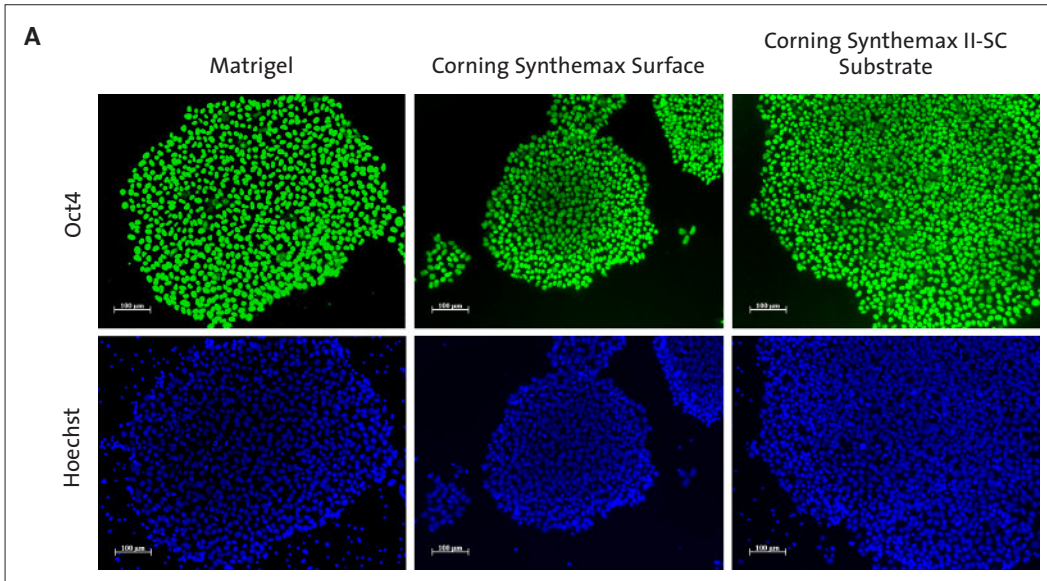


Figure 2. hiPSC morphology at p1 and p7 on Matrigel coated surface, Corning Synthemax Surface, and Corning Synthemax II-SC Substrate coated surface in mTeSR1 defined medium. Typical hiPSC morphology, including high nuclear-to-cytoplasm ratio, well-defined colony borders, tight colony centers, and a low population of differentiated cells, was observed across all surfaces.

A



B

	% Positive Cells		
hiPSC Marker	**Matrigel™**	**Corning Synthemax Surface**	**Corning Synthemax II-SC Substrate**
Oct4 (n=5)	81 ±7	86 ±4	85 ±7
SSEA4 (n=3)	86 ±5	92 ±3	91 ±2

Figure 3. Expression of Oct4 and SSEA4 pluripotency markers in hiPSC during seven passages on different surfaces in mTeSR1 medium. (A) Oct4 immunofluorescent staining (top) and Hoechst nuclear staining (bottom) is shown for representative fields on Matrigel, Corning Synthemax Surface, and Corning Synthemax II-SC Substrate at 10x magnification. (B) The average values for Oct4 and SSEA4 positive cells during multiple passages were measured by flow cytometry analyses. Cells grown on all 3 surfaces demonstrated comparable pluripotency marker expression.

population. Colonies on Corning® Synthemax® II-SC Substrate coated surface and Matrigel™ coated surface were slightly larger and less compact compared to those on Corning Synthemax Surface (Fig. 2 and 3A). Consistent cell morphology was maintained throughout the multi-passage study on all surfaces.

In addition, hiPSC cultured on all 3 surfaces maintained high levels of expression of pluripotentcy markers Oct4 and SSEA4 during 7 sequential passages in mTeSR1 medium (Fig. 3). Immunofluorescent (IF) staining for Oct4 marker shown in Fig. 3A indicates that the majority of cells express Oct4 marker (green color) when compared to the total cell population by nuclear staining (blue color). Consistent with IF staining, quantitative flow cytometry analyses showed higher than 80% expression of both Oct4 and SSEA4 markers for cells cultured on all 3 surfaces (Fig. 3B).

Importantly, hiPSC maintained normal karyotype (46XX) after multiple passages on Corning Synthemax II-SC Substrate coated surface (Fig. 4).

Conclusions

- Corning Synthemax II-SC Substrate coated surface supports the attachment and multi-passage expansion of hiPSC in defined medium.
- Cells cultured on Corning Synthemax II-SC Substrate coated surface maintain consistent doubling time, typical colony morphology, high expression of pluripotency markers, and normal karyotype.

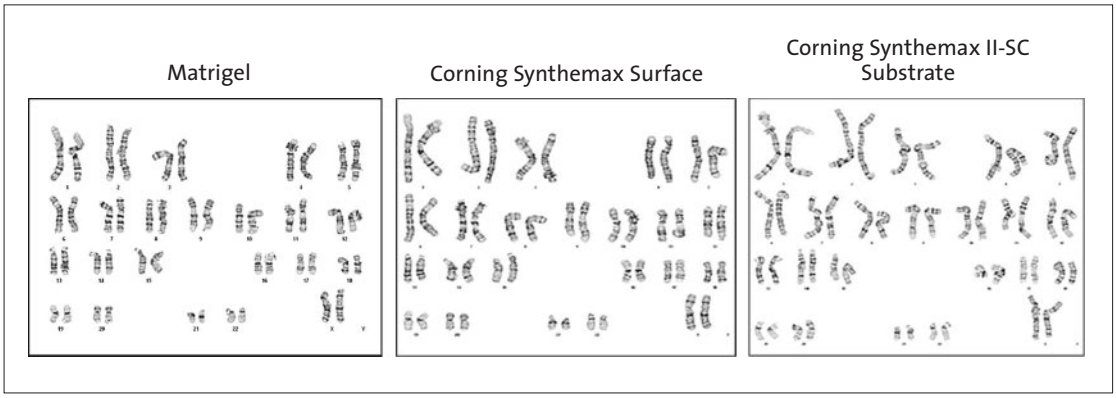


Figure 4. Karyotype of hiPSC after multiple passages on different surfaces. Normal karyotype (46XX) was observed for passage 6 cells on all surfaces.

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