Culturing hESCs on the Corning® Synthemax™ Surface

Protocol

Introduction

In much of today's stem cell research, there is a growing need for cell culture surfaces to support the specific needs of hESC cultures. Current methods to maintain hESCs in an undifferentiated state often employ mouse feeder layers or biological substrates. These methods can be problematic because they are difficult to work with, can be inconsistent, and introduce the possibility of contamination from the animal source. To this end, Corning scientists have developed a novel, non-biological surface for the culture of hESCs. The Corning Synthemax Surface offers researchers an alternative growth surface which does not require special storage or handling conditions. The following protocol describes maintenance of human embryonic stem cells H1 and H7 WiCell® lines on the Corning Synthemax Surface using a chemically defined medium. Due to differences in how various stem cells are maintained, it is highly recommended that researchers optimize the growth conditions based on their individual cell lines. Results may vary depending on cell line used, medium condition, cell seeding density, cell dissociation technique, etc.

It is highly recommended to read entire protocol before beginning.

Materials

The original source for materials listed below had been modified. Sigma-Aldrich alternatives have been posted wherever applicable. Equivalent reagents from preferred vendors may be used.

Corning Synthemax-R Surface 6 well plates (CLS3978XX1 or CLS3979XX1)
Alternatively, Corning Synthemax Surface 6 well plates (CLS3876XX1 or CLS3877XX1), T-75 flasks (CLS3972XX1) or T-225 flasks (CLS3977XX1)
hES cell lines, such as H1 and H7 WiCell lines
Human basic fibroblast growth factor (hbFGF) (Sigma-Aldrich alternative, F0291)
Human transforming growth factor -β1 (hTGF-β1) (Sigma-Aldrich Alternative - recombinant, from CHO cells, T7039, recombinant, from HEK-293 cells, H8541)
Dulbecco's phosphate buffered saline (D-PBS) without Ca2+ or Mg2+ (Sigma-Aldrich alternative – D8537)
X-VIVO™ 10 basal medium (Sigma-Aldrich alternative – Stemline T Cell Medium, S1694, and Hematopoietic Medium, S0192)
1M hydrochloric acid (HCl) (Sigma-Aldrich alternative – H9892)
Human serum albumin (HSA) (Sigma-Aldrich alternative – A6909)
200 U/mL collagenase IV (Sigma-Aldrich Alternatives – Collagenase Typer V, Sterile filtered, suitable for release of physiologically active rat pancreatic islets, C2014)
KnockOut™ DMEM (KO-DMEM) Sigma-Aldrich Alternatives - See Nutri-stem Media at www.sigmaaldrich.com, Products available June 2011)
0.02% EDTA (Sigma® Cat. No. E8008) or other cell dissociation solution
Fetal bovine serum (FBS) (Sigma-Aldrich alternative – F2442, batch qualified)
Plastic scraper, small (CLS3010) or large (CLS3011)
0.22 μM filter units (CLS430767)
50 mL centrifuge tubes (CLS430290)
5 mL aspirating pipets (CLS9099)
150 mL storage bottle (CLS431175)
250 mL storage bottle (CLS430281)

Working Solution and Media Preparation

All work should be done under sterile conditions using proper aseptic technique.
hbFGF Working Solution: prepare on ice; make an 8 μg/mL working solution by making 1:X dilution in D-PBS (calculate X based on the stock concentration).
hTGF-b1 Buffer: prepare at 4 mM HCl in D-PBS with 0.1% HSA.

hTGF-b1 Working Solution: prepare on ice; make 1 μg/mL working solution by making 1:X dilution in hTGF-β1 buffer (calculate X based on the stock concentration).
Collagenase IV: reconstitute in KO-DMEM (other basal medium can be used) to the final concentration of 200 U collagenase/mL, and filter through a 0.22 μM filtration system.
Growth Medium: X-VIVO™ 10 medium supplemented with 0.5 ng/mL TGFβ1 and 80 ng/mL hbFGF, and sterile filtered through a 0.22 μM filter system.

hES Cell Culture on Corning® Synthemax™ Surface

Thawing hESCs

To thaw hES cells, follow the suggested instructions or protocols provided by the cell line providers. Each hES cell line has its optimum thawing procedure and should be followed accordingly.

Recommended Passaging/Expanding of hESCs

Optimal seeding densities will vary from one cell line to another. Also, media conditions will determine proper seeding densities. Predetermine the best conditions to be used in your system. Table 1 gives suggested seeding densities based on internal work done by the Life Sciences Division of Corning Incorporated, using the H1 and H7 cell lines.
hESC cultures are ready to passage when hESC colonies cover approximately 80% of the culture surface (Fig. 1F). With recommended seeding densities and media conditions, most cell lines will reach 80% confluence within 4 to 6 days.
If cell counts are necessary, we recommend setting up a control well or flask and using it to perform cell counts and viability testing. These counts can then be used as reference point for the remaining cultures. If simply maintaining and passaging cells, please skip to "Passaging Cells" section below.

Harvesting to Obtain Cell Count

1. Pre-warm growth medium, collagenase IV, and EDTA (or other cell dissociation solution) to 37°C.
2. Aspirate culture medium from a Corning Synthemax Surface vessel.
3. Add pre-warmed Collagenase IV and incubate at 37ºC for 2 to 3 minutes.
4. Aspirate off Collagenase IV.
5.Wash cells with D-PBS, aspirate. See Table 2 for recommended volumes.
6. Add warmed EDTA (or dissociating solution) and incubate at 37ºC for 10 minutes.
7. Pipette cells in EDTA 3 to 5 times, then transfer to a 150 mL bottle containing FBS.

Table 1. Surface Area and Recommended Seeding Densities

	Growth Area (cm^2)	Media Volume (mL)	H1 (cell/cm^2)	H7 (cells/cm^2)
6 well plate	9.5/well	4	~1 x 10^5	~1 x 10^5
T-75 flask	75	25	~1 x 10^5	~1 x 10^5
T-225 flask	225	75	~1 x 10^5	~1 x 10^5

Table 2. Recommended Reagent Volumes for Cell Harvesting

	Collagenase IV (200 U/mL)	1st D-PBS Wash (mL)	FBS (mL)	EDTA (mL)	2nd D-PBS Wash (mL)
6 well plate (per well)	1 mL	3	0.5	1	1
T-75 flask	5 mL	10	2	5	5
T-225 flask	15 mL	30	6	15	15

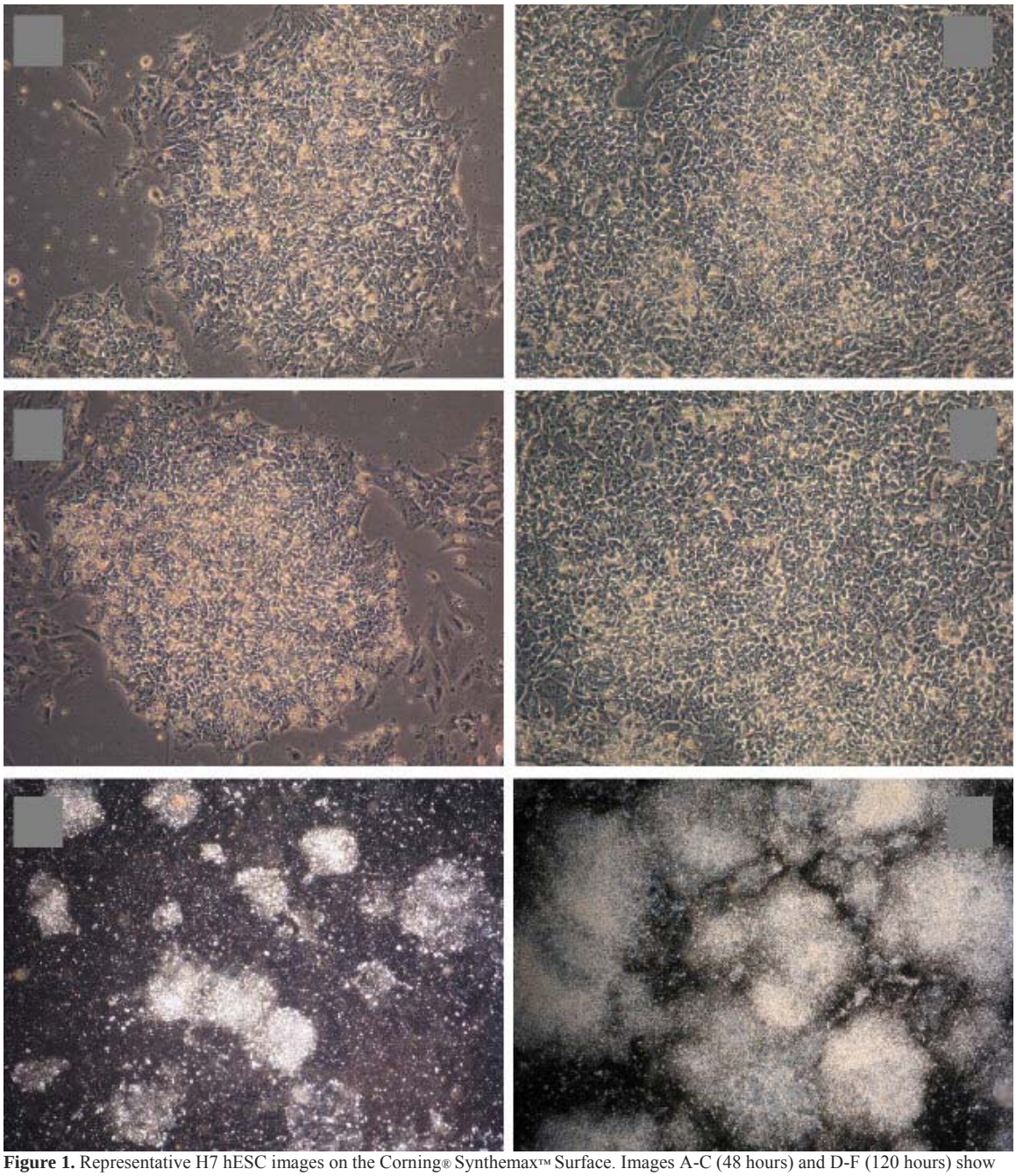

Figure 1. Representative H7 hESC images on the Corning® Synthemax™ Surface. Images A-C (48 hours) and D-F (120 hours) show typical morphology of hES cells grown on Corning Synthemax Surface in growth medium. A, B, D and E were obtained with 40x magnification. Figures C and F were obtained with 10x magnification.

8. Wash with D-PBS. Pipette 3 to 5 times and transfer to the 150 mL bottle. Repeat D-PBS rinse.
9. Pipette cell mixture 3 to 5 times to assure single-cell suspension and dilute appropriately for cell count (10 mL for one well of a 6 well plate; 40 mL for a T-75 flask; 120 mL for T-225 flask).
10. Count cells and calculate total cell number.

Passaging Cells

1. Aspirate spent medium from remaining wells of the 6 well plate, T-75 or T-225 flasks.
2. Add pre-warmed collagenase IV and incubate at 37ºC for the same length of time as the count controls using the same volume.
3. Aspirate off collagenase IV.
4. Wash cells with D-PBS. See Table 3 for recommended volumes. Aspirate off.

5. Add pre-warmed growth medium and use a cell scraper to gently remove cells from surface. See Table 3 for recommended volumes.

Table 3. Recommended Reagent Volumes for Cell Passaging

	Collagenase IV (200 U/mL)	D-PBS Wash (mL)	Growth Medium (mL)	Growth Medium Wash (mL)
6 well plate (per well)	1 mL	3	1	1
T-75 flask	5 mL	10	5	5
T-225 flask	15 mL	30	15	15

6. Transfer medium with cells into a 50 mL centrifuge tube (for 6 well plate) or 150 mL bottle (for T-75 or T-225 flasks).
7. Rinse vessels with growth medium and transfer rinse to a tube/bottle containing cell collection.
8. Gently triturate clumps a few times by pipetting up and down to achieve the clump size of approximately 0.5 to <1 mm in diameter. Avoid making single-cell suspension.
9. Distribute equal number of cells into each well of a 6 well plate, T-75 or T-225 flask using optimized cell seeding density determined for your system.
10. Recommended final volume of growth medium is listed in Table 1.
11. Place newly seeded vessels in 5% CO2, humidified, 37ºC incubator being careful to ensure an even cell distribution.

Media Changes/Feeding

Perform a complete media change 48 hours after seeding, then change media daily. The medium exchange schedule can vary for different cell lines and media conditions. Refer to Table 1 for media volumes.

For additional product or technical information, please visit
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