Culturing hESCs on the Corning® Synthemax™ Surface

Protocol

CORNING

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

- Corning Synthemax-R Surface 6 well plates (Corning Cat. No. 3978 or 3979)
- Alternatively, Corning Synthemax Surface 6 well plates (Corning Cat. No. 3876 or 3877), T-75 flasks (Corning Cat. No. 3972) or T-225 flasks (Corning Cat. No. 3977)
- hES cell lines, such as H1 and H7 WiCell lines
- Human basic fibroblast growth factor (hbFGF) (R&D Systems® Cat. No. 234-FSE/CF)
- Human transforming growth factor -β1 (hTGF-β1) (R&D Systems Cat. No. 240-B)
- Dulbecco's phosphate buffered saline (D-PBS) without Ca^{2+} or Mg^{2+} (Invitrogen™ Cat. No. 14190-144)
- X-VIVO™ 10 basal medium (Lonza Cat. No. 04-743Q)
- 1M hydrochloric acid (HCl) (J.T.Baker® Cat. No. 9544)
- Human serum albumin (HSA) (Grifols Cat. No.TS1-165)
- 200 U/mL collagenase IV (Invitrogen Cat. No. 17104)
- KnockOut™ DMEM (KO-DMEM) or other basal medium (Invitrogen Cat. No. 10829-018)
- 0.02% EDTA (Sigma® Cat. No. E8008) or other cell dissociation solution
- Fetal bovine serum (FBS) (Invitrogen Cat. No. 16000-077)
- Plastic scraper, small (Corning Cat. No. 3010) or large (Corning Cat. No. 3011)
- 0.22 µM filter units (Corning Cat. No. 430767)
- 50 mL centrifuge tubes (Corning Cat. No. 430290)
- 5 mL aspirating pipets (Corning Cat. No. 9099)
- 150 mL storage bottle (Corning Cat. No. 431175)
- 250 mL storage bottle (Corning Cat. No. 430281)

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-β1 Buffer: prepare at 4 mM HCl in D-PBS with 0.1% HSA.
- hTGF-β1 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 Corning Life Sciences 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 37°C for 10 minutes.
7. Pipette cells in EDTA 3 to 5 times, then transfer to a 150 mL bottle containing FBS.
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.

Table 1. Surface Area and Recommended Seeding Densities

	Growth Area (cm^2)	Media Volume (mL)	H1 (cells/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

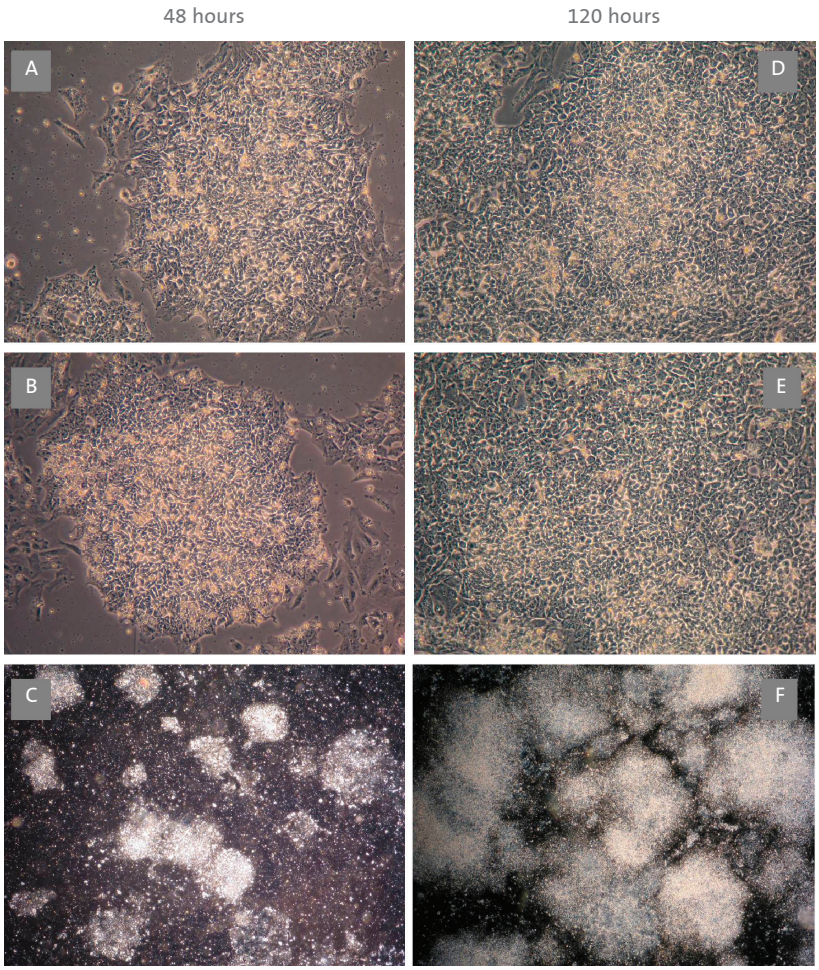

Figure 1. Representative H7 hESC images on the Corning® Synthemax™ Surface. Images A-C (48 hours) and D-F (120 hours) show typical morphology of hES cells grown on Corning Synthemax Surface in growth medium. A, B, D and E were obtained with 40x magnification. Figures C and F were obtained with 10x magnification.

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.
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.

Table 3. Recommended Reagent Volumes for Cell Passaging

	Collagenase IV (200 U/mL)	D-PBS Wash (mL)	Growth Medium (mL)	Growth Media 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

10. Recommended final volume of growth medium is listed in Table 1.
11. Place newly seeded vessels in 5% CO_2, 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 more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional product or technical information, visit **www.corning.com/lifesciences**, or contact our Scientific Support Team at **ScientificSupportEMEA@corning.com**.

Corning Incorporated
Life Sciences Europe
Corning BV
Fogostraat 12
1060 LJ Amsterdam
The Netherlands
Phone: +31 (0) 20 659 60 51
Fax: +31 (0) 20 659 76 73
CSEurope@corning.com
www.corning.com/lifesciences

Support Offices

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

CORNING | FALCON AXYGEN GOSSELIN

© 2011 Corning Incorporated Printed in Europe 10/11 CLS-AN-148-REV5-A4 PN-133876001