The Corning® Synthemax™ Surface: A Synthetic, Xeno-Free Surface For Long-Term Self-Renewal of Human Embryonic Stem Cells in Defined Media

Jennifer L. Weber[1], David M. Weber[1], Andrei Fadeev[1], Yue Zhou[1], Paula Dolley-Sonneville[1], Jessica J. Kelley[1], Jiwei Yang[2], Liqun Qiu[2], Catherine Priest[2], Christopher Shogbon[1], Arthur Martin[1], Jodelle Nelson[1], James Beltzer[1], Santona Pal[1], Anthony Davies[2], Ralph Brandenberger[2], Zara Melkoumian[1].

[1]One Science Center Drive, Corning Life Science, Corning Incorporated, Corning, NY 14831, USA
[2]Geron Corporation, 230 Constitution Drive, Menlo Park, CA 94025, USA

Introduction

Scalable, reproducible, low cost and regulatory-friendly technologies must be developed to enable clinical use of human embryonic stem cell (hESC)-based therapeutics. hESC culture methods use complex, animal-derived products, such as mouse feeder layers, Matrigel™, murine laminin or human-derived biological substances as surfaces to which the hESCs attach. Most of these materials are costly, of limited scalability, have batch to batch variability and are a potential source of adventitious agents. For clinical application of hESC-based therapeutics it is highly desirable to have defined, scalable culture systems for production of cells suitable for clinical use.

In this study we describe the development of a fully synthetic, xeno-free surface for the culture of undifferentiated hESC. A peptide sequence derived from the active domain of the vitronectin protein was covalently linked to a synthetic acrylate polymer surface to mimic biological ligands for cell adhesion. Self-renewal and pluripotency of multiple hESC lines (H7, H1, H9, and BG01v) cultured on Synthemax Surface was compared to cells grown on Matrigel control surfaces under various defined media conditions (STEMPRO®, NutriStem™, mTeSR®1, and XVIVO™ 10).

Our results demonstrate efficient adhesion and self-renewal of H7, H1, H9 and BG01v/hOG hESCs on Synthemax Surface for up to 20 serial passages in defined media. Importantly, stable proliferation rate, expression of stem cell specific markers (Oct-4, TRA 1-60, SSEA-4), in vitro and in vivo pluripotency and normal karyotype were retained throughout multiple passages on Synthemax Surface. Further, we demonstrated successful scale-up of Synthemax Surface to large culture vessel formats to accommodate the clinical scale production of therapeutic cells.

To our knowledge, Synthemax Surface is the only commercially available, synthetic, non-biological surface that supports the long-term, multi-passage expansion of undifferentiated hESC in chemically-defined, xeno-free media. We believe Synthemax Surface will be applicable for both research purposes and scalable manufacturing of hESC-derived cellular therapeutics.

Methods

- **Cells**: H7, H1, H9 and BG01v hESC
- **Media conditions**:
  - X-VIVO™10 (Lonza, Cat No. 04-743Q) (with 80ng/ml bFGF + 0.5ng/ml TGFb1)
  - mTeSR®1 (Stem Cell Technologies, Cat. No. 05850)
  - NutriStem™ (StemGent, Cat. No. 01-0005)
  - STEMPRO® hESC SFM (Invitrogen, Cat. No. A1000701)

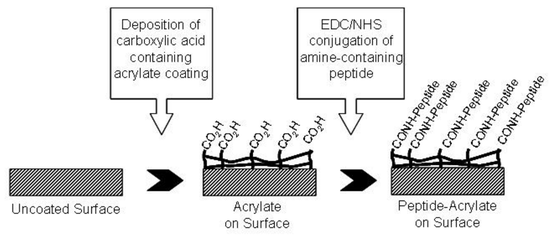
  NOTE: X-VIVO 10 and NutriStem are xeno-free, defined media. mTeSR1 and STEMPRO are defined media.
- **Surfaces:**
  - Synthemax™ Surface (6-wp, Corning, Cat. No. 3877XX1) (T75 flasks, Corning, Cat. No. 3973XX1)
  - Biological coating: GFR-Matrigel™ (BD Biosciences, Cat. No. 356231) Geltrex™ (Invitrogen, Cat. No. A10480)
  - Tissue Culture Treated (TCT) (6-wp, Corning, Cat. No. 3516)
- **hESC sub-cultivation method:**

  H7, H1, H9 and BG01v hESC were cultured on Matrigel-coated TCT plastic (1:30 dilution in KnockOut ™ DMEM) in chemically-defined medium. Cultures were passaged every 4-6 days, as cells become about 80% confluent, by incubation with 200 U/ml collagenase IV, followed by brief DBPS wash and gentle scraping. Seeding density was about 100,000 cells/cm^2. Cells received fresh medium every day, except for the day after split. Cultures were routinely examined by flow cytometery for the expression of hESC markers, and cytogenetic analysis by G-banding for normal karyotype.
- **hESC long-term expansion:**

  For each passage, H7, H1, H9, and BG01v cells were sub-cultured onto Synthemax Surface or biological control 6-well plates at a density of 100,000 cell/cm^2. Cultures were fed every day, except for the day after split. Microscopic examination of cell and colony morphology was performed daily. Cell viability and number were assessed at the end of each passage, by harvesting duplicate samples with collagenase IV/EDTA treatment followed by cell counting with automated cell number/viability analyzer, Vi-Cell™ (Beckman Coulter). Expression of hESC markers was assessed by flow cytometry at the end of each passage. To monitor genomic integrity, cell samples for all experimental conditions were submitted for karyotyping analysis by G-banding (Cytogenetics laboratories) at passages 0, 5, and 10. H7 hESC were routinely maintained in X-VIVO 10 medium on Matrigel. For the experiment, cells were seeded at 1 x 10^6 cells per well of a 6-well plate (9.5cm^2) and expanded in either STEMPRO or NutriStem. No pre-conditioning of cells to these media was performed.

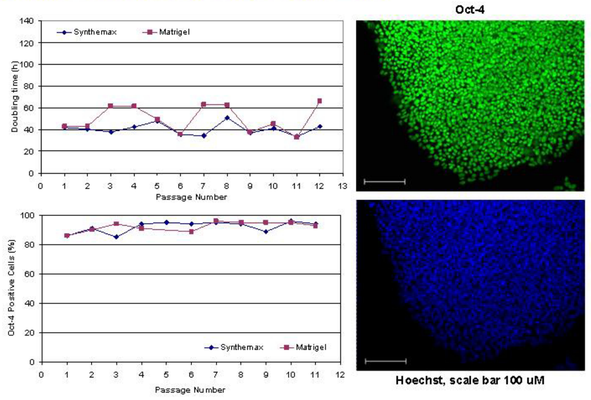
Results

Figure 1: Schematic representation of Synthemax Surface coating process.



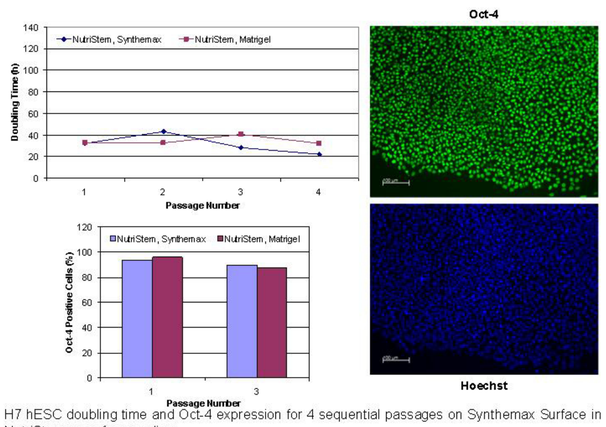
Cell cultureware is coated with an acrylate layer, and EDC/NHS chemistry is used to attach cell adhesive peptide.

Figure 2: H7 hESC long-term expansion on Synthemax Surface in X-VIVO 10 xeno-free medium.



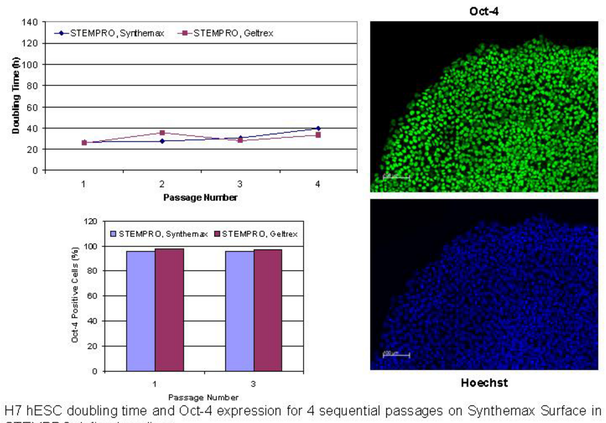
H7 hESC doubling time and Oct-4 expression for 12 sequential passages on Synthemax Surface in X-VIVO 10 xeno-free medium. Similar results were seen in T75 flasks.

Figure 3: H7 hESC long-term expansion on Synthemax Surface in NutriStem xeno-free medium.



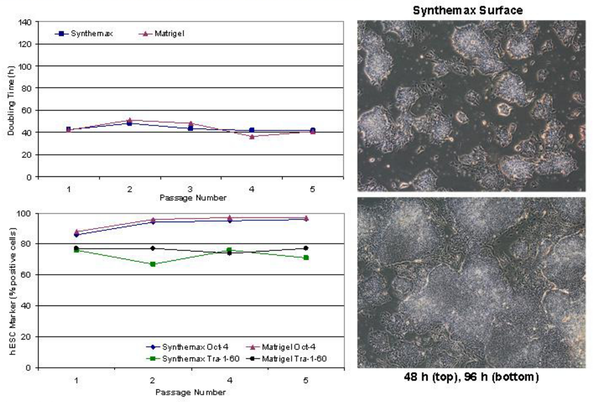
H7 hESC doubling time and Oct-4 expression for 4 sequential passages on Synthemax Surface in NutriStem xeno-free medium.

Figure 4: H7 hESC long-term expansion on Synthemax Surface in STEMPRO defined medium.



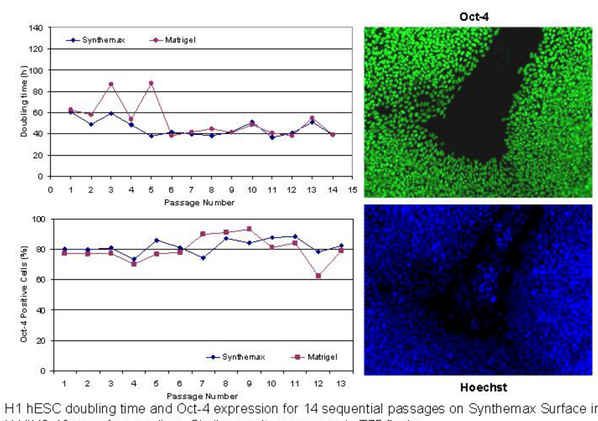
H7 hESC doubling time and Oct-4 expression for 4 sequential passages on Synthemax Surface in STEMPRO defined medium.

Figure 5: H9 hESC long-term expansion on Synthemax Surface in mTeSR1 defined medium.



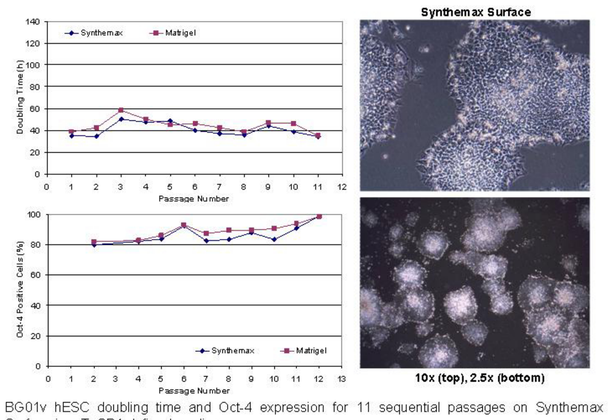
H9 hESC doubling time and phenotypic marker expression for 5 sequential passages on Synthemax Surface T75 in mTeSR1 defined medium.

Figure 6: H1 hESC long-term expansion on Synthemax Surface in XVIVO-10 xeno-free medium.



H1 hESC doubling time and Oct-4 expression for 14 sequential passages on Synthemax Surface in X-VIVO 10 xeno-free medium. Similar results were seen in T75 flasks.

Figure 7: BG01v hESC long-term expansion on Synthemax Surface in mTeSR1 defined medium.



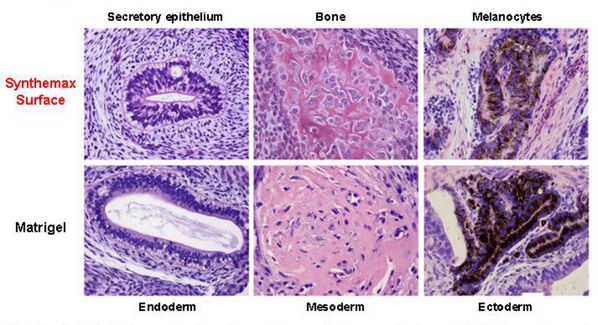
BG01v hESC doubling time and Oct-4 expression for 11 sequential passages on Synthemax Surface in mTeSR1 defined medium.

Table 1: Summary of hESC long-term expansion on Synthemax Surface in defined media.

Cell Line	Culture Media	Surface	Doubling Time (h)	% Oct-4 + cells	% Tra 1-60 + cells	% SSEA-4 + cells
H7	X-VIVO 10	Synthemax	41±5	92±4	85	100
		Matrigel	50±12	92±3	96	100
	mTeSR1	Synthemax	55±21	90±4	66	-
		Matrigel	69±26	83±8	56	-
	STEMPRO	Synthemax	31±6	96±0	-	-
		Geltrex	31±5	97±0	-	-
	NutriStem	Synthemax	32±9	92±3	-	-
		Matrigel	35±4	92±6	-	-
H1	X-VIVO 10	Synthemax	46±8	82±5	78±4	91
		Matrigel	53±16	80±8	83±6	84
H9	mTeSR1	Synthemax	43±3	93±5	73±4	-
		Matrigel	44±6	95±4	76±2	-
BG01v	mTeSR1	Synthemax	40±6	86±6	-	-
		Matrigel	44±6	89±5	-	-

Doubling time and phenotypic marker expression for long-term hESC expansion on Synthemax Surface in chemically-defined media.

Figure 8: hESCs retain pluripotency after long term culture on Synthemax Surface in defined media.



H7 hESC retain pluripotency after long-term culture on Synthemax Surface. H7 hESC were thawed and cultured for 8 passages on Synthemax and Matrigel. Cells were harvested and injected by intramuscular injection in the flank of SCID/bg mice. Tissues from all three germ layers, represented as secretory epithelium (endoderm), cartilage (mesoderm) and neuroepithelium (ectoderm), were identified in teratomas formed by cells cultured on Synthemax Surface or Matrigel. In addition, pluripotency of H7 hESCs maintained on Synthemax and Matrigel was confirmed by embryoid body-mediated differentiation *in vitro*.

Conclusions

- Synthemax Surface supports the long-term, multi-passage expansion of undifferentiated H7, H1, H9 and BG01v hESC in several commercially-available defined media.
- hESCs expanded on Synthemax Surface retain consistent doubling time, phenotypic marker expression, karyotype, and *in-vivo* pluripotency.
- The Synthemax Surface in combination with chemically defined media enables scalable culture of hESC for therapeutic applications.

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