CORNING

Corning® Synthemax™ Surface: a tool for feeder-free, xeno-free culture of human embryonic stem cells

Currently, human embryonic stem cells (hESCs) are cultured on feeder cells or complex mixtures of proteins extracted from mouse tumors[1,2]. To allow commercialization of hESC-derived therapeutic cells, culture methods are required that are robust and scalable and that use chemically defined, xeno-free materials. Corning Synthemax Surface is a novel synthetic surface that permits consistent long-term self-renewal of multiple hESC lines in defined, xeno-free media and differentiation of cells to functional cardiomyocytes[3].

Synthemax Surface technology

Synthemax Surface is a unique synthetic surface composed of RGD-containing short peptides covalently immobilized on acrylate coating to mimic the natural cell environment. The surface is manufactured under current Good Manufacturing Practices (cGMP) using a proprietary surface coating technology and defined, xeno-free materials, providing lot-to-lot consistency. Culture vessels with Synthemax Surface are ready to use and do not require any additional preparation by the user. Synthemax Surface is terminally sterilized by gamma irradiation, eliminating the risk of pathogen contamination associated with biological surfaces. Unlike biological surfaces that require special storage conditions and have a limited shelf life, Synthemax Surface is stable at room temperature for at least two years.

Synthemax Surface supports long-term self-renewal of hESCs in xeno-free medium

The ability of Synthemax Surface to support long-term self-renewal of hESCs was evaluated by serial passaging of H7 hESCs on Synthemax Surface in defined, xeno-free medium (X-VIVO™ 10 basal medium supplemented with 80 ng/ml hrbFGF and 0.5 ng/ml hrTGF-β (X-VIVO10+GF medium)). As shown in **Figure 1a**, Synthemax Surface supported a stable proliferation rate of H7 hESCs (doubling time of 41 ± 5 h) for 12 serial passages. Importantly, cells retained normal karyotype at the end of 12 passages (**Fig. 1b**). hESC-specific phenotypic markers, such as Oct4 and SSEA4, were evaluated qualitatively by indirect immunofluorescence staining (**Fig. 2a**,**b**) and quantitatively by flow cytometry (**Fig. 2c**,**d**). After ten passages on Synthemax Surface in X-VIVO10+GF medium, H7 hESCs retained high levels of both markers, suggesting their undifferentiated status. Similar results for Synthemax Surface were demonstrated with other hESC lines and defined medium conditions[3].

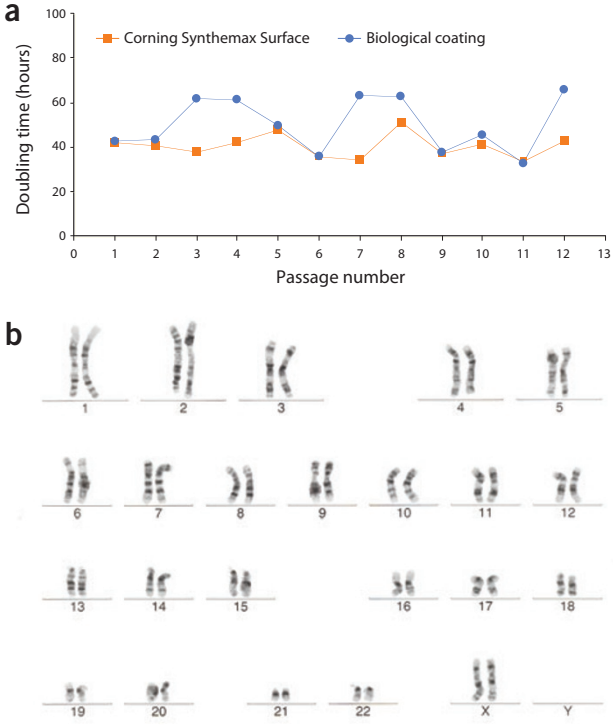

Figure 1 | H7 hESCs maintained on Synthemax Surface in defined, xeno-free medium show a stable proliferation rate and normal karyotype. H7 hESCs cultured on Synthemax Surface for 12 serial passages in X-VIVO10+GF medium demonstrate more consistent doubling time relative to biological coating (**a**) and retain normal karyotype, as determined by G-banding analysis (**b**).

Jennifer L Weber[1], Paula Dolley-Sonneville[1], David M Weber[1], Andrei G Fadeev[1], Yue Zhou[1], Jiwei Yang[2], Catherine A Priest[2], Ralph Brandenberger[2] & Zara Melkoumian[1]

[1]Corning Incorporated, Life Sciences, Corning, New York, USA.
[2]Geron Corporation, Menlo Park, California, USA.
Correspondence should be addressed to Z.M. (melkoumiz@corning.com).

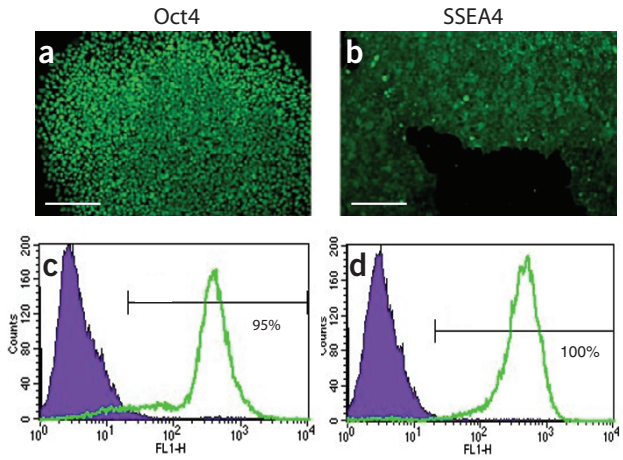

Figure 2 | H7 hESCs retain phenotypic markers after ten passages on Synthemax Surface. Indirect immunofluorescent staining of H7 hESCs for Oct4 (**a**) and SSEA4 (**b**) markers after ten serial passages on Synthemax Surface in X-VIVO10+GF medium; scale bar, 100 µm. Flow cytometry histograms demonstrate a high proportion of H7 hESCs expressing Oct4 (**c**) and SSEA4 (**d**) markers after ten passages on Synthemax Surface.

hESCs retain pluripotency after long-term culture on Synthemax Surface

Pluripotency, the ability to differentiate into cells of all three germ layers, is a fundamental property of hESCs. This property is a critical parameter when evaluating new culture conditions for hESCs. As shown in **Figure 3a**, H7 hESCs maintained on Synthemax Surface for ten serial passages were able to differentiate into cells of all three germ layers when injected into immunodeficient mice, confirming their pluripotent status.

These results suggest that H7 hESCs can be successfully propagated on Synthemax Surface for multiple passages in defined, xeno-free medium with a stable proliferation rate and normal karyotype, while retaining hESC-specific phenotypic marker expression and pluripotency.

Synthemax Surface supports differentiation of hESCs to cardiomyocytes

For therapeutic applications of hESCs, it is highly desirable to have defined culture conditions for both the expansion and the differentiation phases of therapeutic cell production. Therefore, H7 hESCs cultured on Synthemax Surface for ten passages were differentiated to cardiomyocytes on the same surface using a directed differentiation protocol[3]. As demonstrated by immunofluorescence staining in **Figure 3b**, Synthemax Surface supports differentiation of

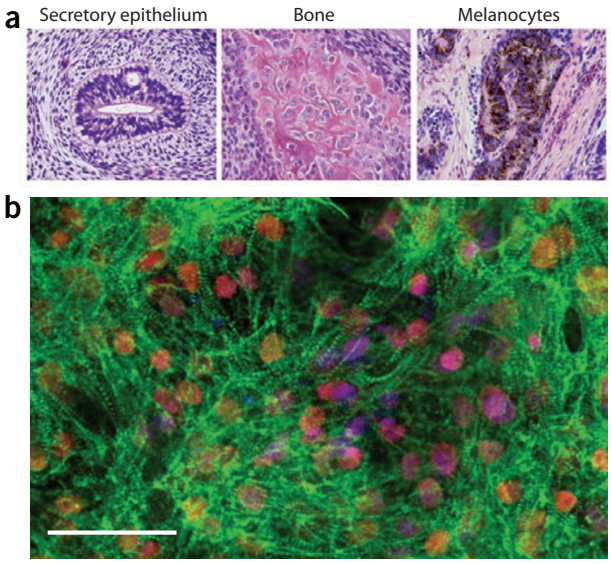

Figure 3 | H7 hESCs retain pluripotency and the ability to differentiate into cardiomyocytes after ten passages on Synthemax Surface. (**a**) Positive staining for representative tissues from all three germ layers—secretory epithelium (endoderm), bone (mesoderm) and melanocytes (ectoderm)—in teratomas developed after injection of mice with H7 hESCs cultured on Synthemax Surface for ten passages. (**b**) Confocal microscopy image of cardiomyocytes derived from H7 hESCs on Synthemax Surface after indirect immunofluorescent staining for cardiomyocyte-specific markers: Nkx2.5 (red), α-actinin (green) and nuclear stain (blue). Scale bar, 50 µm.

H7 hESCs into cardiomyocytes expressing cardiomyocyte-specific markers α-actinin and Nkx2.5. Additional quantitative assessment of differentiation by flow cytometry and electrophysiology is provided in ref. 3.

In conclusion, Synthemax Surface provides a xeno-free solution for long-term self-renewal of hESCs and directed differentiation of hESCs to cardiomyocytes in a defined medium. We believe Synthemax Surface will be useful for both research purposes and production of hESC-derived cells for cellular therapies.

1. Amit, M. *et al.* Human feeder layers for human embryonic stem cells. *Biol. Reprod.* **68**, 2150–2156 (2003).
2. Xu, C. *et al.* Feeder-free growth of undifferentiated human embryonic stem cells. *Nat. Biotechnol.* **19**, 971–974 (2001).
3. Melkoumian, Z. *et al.* Synthetic peptide-acrylate surfaces for long-term self-renewal and cardiomyocyte differentiation of human embryonic stem cells. *Nat. Biotechnol.* **28**, 606–610 (2010).