Synthetic Interfaces for the Long-term Self-Renewal of Human Embryonic Stem Cells

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Introduction: Long-term self-renewal of human embryonic stem cells (hESCs) requires the use of isolated animal matrix proteins such as Matrigel™, a basement membrane isolated from a mouse sarcoma. Matrigel™ has considerable lot-to-lot variability and potential for xenogenic contamination [1]. For more widespread clinical use, there is a need for a chemically defined, low cost, scalable culture system for the growth and expansion of hESCs in serum-free, and chemically-defined media. Until now, only short-term culture has been demonstrated on fully defined substrates in chemically defined media [2-4]. We have created a biointerface that supports the long-term growth of hESCs in chemically-defined media.

Methods: *APMAAm Polymerization.* Tissue culture plates were activated by oxygen plasma and a solution of N-(3-Aminopropyl)methacrylamide hydrochloride (APMAAm), *N*,*N*-methylenebis(acrylamide), and Irgacure 2959 (Ciba) was photo-polymerized directly onto the wells for 1 min using a UV light source. *Cell Culture.* hESC lines H1 and H9-Oct4-GFP (Wicell) were cultured on APMAAm and Matrigel controls in chemically defined mTESR™1 media [5]. Cells were fed daily and passaged 1:3-1:6 every 3-5 days by exposure to Collagenase IV and scraping. For embroid body (EB) formation, cells were passaged into 20% FBS in KODMEM for 15 days. For cell attachment experiments, basal (incomplete) mTESR™1 media was supplemented with bovine serum albumin (BSA), transforming growth factor β (TGF-β), or basic fibroblast growth factor (bFGF) at the levels in the complete media.

Results: Human ESCs were cultured on APMAAm surfaces for over 10 passages in chemically-defined mTESR™1 media. Throughout 10 passages, H1s and H9-Oct4-GFPs maintained typical stem cell morphology and grew in colonies similar to cell cultured on Matrigel™(Fig 1a,b). In addition, normal karyotype was confirmed for both cell lines after 10 passages. The pluripotency of both lines was confirmed via immunostaining, where expression levels of pluripotency markers of H1s and H9-Oct4-GFPs were similar to Matrigel™ controls (Fig 1c). In addition, at passage 10, both H1s and H9-Oct4-GFPs on APMAAm showed expression of pluripotency genes consistent with matrigel controls (Fig 1d). H1s and H9-Oct4-GFPs were differentiated into EBs to demonstrate the H1s and H9-Oct4-GFPs were capable of differentiating into all three germ layers *in vitr*o. H1s and H9-Oct4-GFPs cultured on APMAAm and Matrigel™ formed nearly spherical EBs with typical

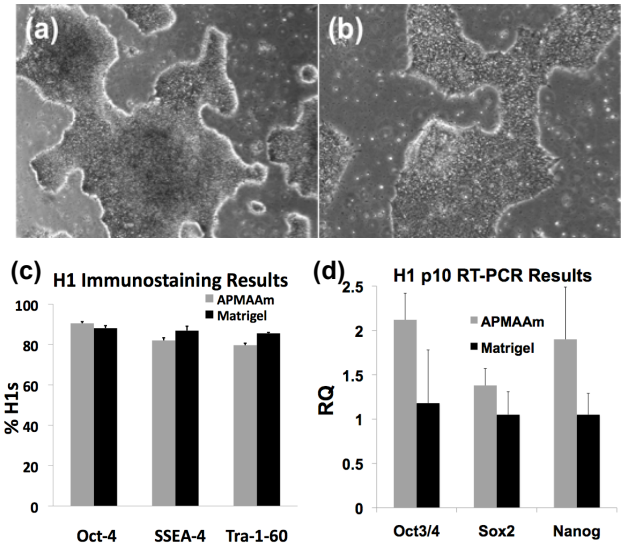

Figure 1. H1s on (a) APMAAm coatings after 10 passages expressed similar levels of pluripotency markers as (b) Matrigel controls as evidenced by (c) immunostaining and (d) RT-PCR.

morphology. At Day 15, qRT-PCR results indicated the formation of all three germ layers in EBs from both APMAAm and Matrigel™ controls. In addition, we explored the role of the proteins in mTESR™1 media responsible for hESC attachment on APMAAm by performing cell attachment studies in incomplete mTESR™1 media supplemented with either BSA, TGF-β or bFGF. mTESR™1 incomplete supplemented with BSA led to the highest amount of cell attachment. In addition, BSA adsorption from mTESR™1 media to the APMAAm coating was observed in real time in a quartz crystal microbalance with dissipation (QCM-D), where a layer of 160 ng/cm^2 of BSA adsorbed onto the APMAAm within 15 min.

Conclusions: We have developed a defined and synthetic culture system that allows for long-term hESCs growth and self-renewal. The primary advantage of this system is that it does not require attachment of peptides to promote cell adhesion, is scalable, and is free of complex, undefined culture conditions.

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