Synthetic surfaces for the culture of human embryonic and adult stem cells

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ABSTRACT

Utility of stem cells for downstream clinical applications primarily rely on the ability to expand cells in large scale under defined media and matrix conditions. To address that need, several chemically defined media systems have been reported for the maintenance and proliferation of pluripotent stem cells. An equally critical component is the use of an extracellular matrix that is animal-origin free and chemically defined.

An ideal synthetic matrix would not only create a microenvironment ideal for stem cell expansion but would also be modular such that it can be adapted to support robust differentiation into lineage of choice. Using a combination of various biomaterials with unique mechanical and thermal properties attached via novel linkers to functional biomolecules, novel coated surfaces can be created. We have evaluated several defined synthetic surfaces in comparison to traditional ECM molecules. Cell based assays were adapted to monitor efficiency of cell attachment to specific surfaces in a high-throughput format. Matrices that supported efficient attachment of ESC were further evaluated for proliferation. ESC expanded on experimental matrices for multiple passages were assessed for pluripotency based on surface marker expression and ability to differentiate into the three representative germ layers. Using the established streamlined process of evaluation, evaluation was extended to novel biomaterials with unique mechanical and thermal properties for 2 and 3 dimensional cell culture.

INTRODUCTION

The generation of human embryonic stem (hES) cells and induced pluripotent stem (iPS) cells has been well-established (1-3). Due to their pluripotent nature, these cells have the potential to revolutionize cell-based therapeutic applications. A key element to realizing this potential is the manner in which these cells are grown and maintained in the undifferentiated state. Many downstream applications require uniform and consistent populations of undifferentiated cells. However, culture-induced variability remains a significant challenge. The original culture systems for hES cells employed basal medium supplemented with fetal calf serum and mouse embryonic fibroblast feeder cell layer support (4). Unknown components from either the serum or the fibroblasts can confound studies to understand the effects of exogenous agents on the growth and differentiation of stem cells. Moreover, these animal products are potential routes of pathogen transmission which presents a significant barrier for clinical applications with stem cells. For these reasons, several chemically defined media systems have been reported for the maintenance and proliferation of pluripotent stem cells (5). An equally critical component is the use of an extracellular matrix that is animal-origin free and chemically defined (6). Here we report the development of a defined xeno-free matrix that supports long-term growth and pluripotency of both embryonic and adult stem cells similar to levels seen using basement membrane extracts (e.g., Geltrex™). CELLstart™, a cGMP manufactured product, is a consistent and scalable surface, that represents a significant advance in understanding and controlling the basic aspects of stem cell biology—a critical step in translating these cells to the clinic.

MATERIALS AND METHODS

All reagents were purchased from Life Technologies, unless otherwise noted.

Cell culture: H9 and BGO1v/hOG human ESCs and CytoTune™ iPSC lines were maintained on irradiated MEFs using hESC media comprised of DMEM/F-12 (Cat # 10565-018), 20% KSR (Cat # 10828-028). Feeder free hESCs and iPSCs were cultured on Geltrex™ (Cat # A1048002) or CELLstart™CTS™ (Cat # A1014201) in StemPro® hESC SFM medium (Cat # 1000701).

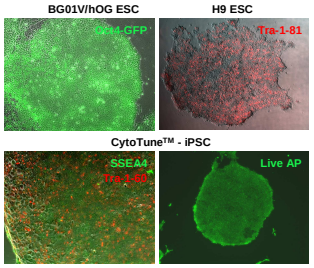
Attachment assay: Feeder free hESCs were harvested using TrypLE™ and triturated to obtain a single cell suspension. hESCs were seeded on a 96 well TC-treated plate with appropriate wells coated with Geltrex™, Attachment factor (Gelatin) (Cat # S-006-100), or CELLstart™CTS™ at given densities in StemPro® hESC SFM. Cell viability was measured using PrestoBlue™ (Cat # 13261) and a fluorometric plate reader.

BacMam labeling and differentiation assays: H9 ESCs were transduced with BacMam 2.0 GFP Control (Cat # B10383) in varying conditions. Embryoid bodies (Ebs) were formed from the GFP labeled ESCs and subjected to undirected differentiation on Geltrex™ coated plates for 1 to 2 weeks. Cultures were fixed and stained with the appropriate antibodies for endoderm, mesoderm, and ectoderm.

Pluripotent characterization: Pluripotency was determined using live immunostaining using directly conjugated antibodies to surface markers: SSEA-4, Tra1-60, and Tra1-81. Alkaline Phosphatase staining was confirmed utilizing a novel LIVE AP fluorescent substrate.

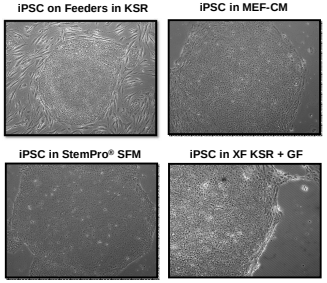
RESULTS

Figure 1. Geltrex™ supports maintenance and expansion of pluripotent stem cells



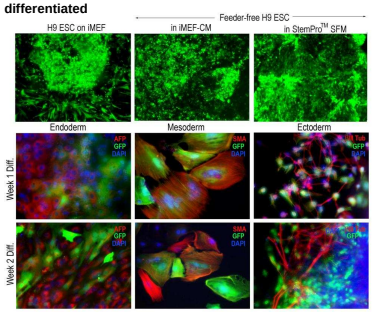
Geltrex™-coated surfaces can be utilized in combination with StemPro® hESC SFM to supports feeder-free culture of ESCs and iPSCs. Cultured cells can be expanded for several generations and continue to express pluripotency markers such as Oct4, Tra-1-60, Tra1-81, SSEA-4 and are alkaline phosphatase positive.

Figure 2. Geltrex™ supports iPSCs in different media systems



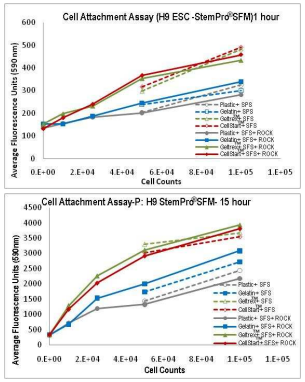
iPSC derived using CytoTune™ or Retro virus (KSR+GF) expanded on Geltrex™ coated dishes in different media systems for feeder-free culture show morphology comparable to cells on feeders.

Figure 3. Geltrex™-coated cells can be labeled and differentiated



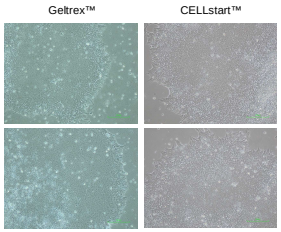
H9 ESCs seeded on Geltrex™ in MEF-CM or StemPro® SFM can be transduced at rates similar to feeder-dependent culture. H9 ESC transduced with BacMam GFP can undergo undirected differentiation, on Geltrex™ coated plates, into the cell types representative of the three germ layers.

Figure 4. Evaluation of H9 ESC attachment on different matrices



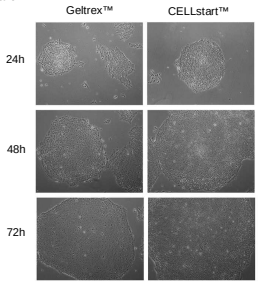
H9 ESC cultured in StemPro® hESC SFM and seeded on Geltrex™ (Green) or CELLstart™ (Red) demonstrate superior attachment and viability as compared to Gelatin (Blue) or TC treated (Gray) surfaces, as measured via 1 hr incubation with PrestoBlue. Cells continue to proliferate and demonstrate healthy metabolic activity after 15 hrs of exposure to PrestoBlue™.

Figure 5. CELLstart™ supports long-term maintenance of ESCs



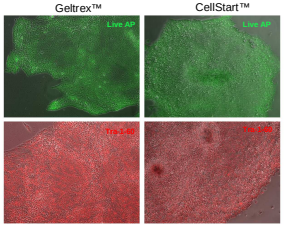
hESCs grown on CELLstart™-coated dishes in StemPro® hESC SFM (passage 4) exhibit normal morphology with compact colonies and defined edges.

Figure 6. Morphology and growth of iPSC on Geltrex™ and CELLstart™



iPSC derived using CytoTune™ were seeded on Geltrex™ or CELLstart™ coated dishes in StemPro® SFM media. Images of cells were collected on Day 1, 2 and 3 and monitored for morphology of cells and growth of the colonies.

Figure 7. Maintenance of pluripotency of iPSC clones on Geltrex™ and CELLstart™



iPSC derived using CytoTune™ were seeded on Geltrex™ or CellStart™ coated dishes in StemPro® SFM media and cells stained with pluripotence markers 72 h post seeding. CellStart™ was able to support attachment growth and pluripotence of iPSC clones. Multi passage experiments on CellStart™ are currently underway to determine long term culture effects.

CONCLUSIONS

- Geltrex™-coated surfaces support the proliferation and maintenance of embryonic and induced pluripotent stem cells over multiple passages as evidenced by the expression of pluripotent markers (e.g., Oct4,Tra-1-81, Tra-1-60, SSEA-4, and alkaline phosphatase)
- iPSC, derived using CytoTune™ or Retrovirus (KSR+GF) and expanded on Geltrex™-coated surfaces across various media systems demonstrate similar morphology of feeder-dependent cultures (e.g., compact colonies, high nucleus/cytoplasm ratio, defined colony edges)
- Geltrex™-coated surfaces support ESC cultures that can be transduced using BacMam at rates similar to feeder-dependent cultures and can be spontaneously differentiated into all three germ lineages (e.g., endoderm, ectoderm, mesoderm)
- CELLstart™, a fully-defined xeno-free substrate supports ESC attachment and growth at levels comparable to Geltrex™ with or without ROCK inhibitor
- CELLstart™ also supports the maintenance of iPSCs with comparable morphology and pluripotence to Geltrex™-coated surfaces
- In combination with a chemically defined media, CELLstart™ represents a fully defined culture system for the growth, maintenance, and differentiation of both embryonic and adult stem cells

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TRADEMARKS/LICENSING