Comparative study of myMATRIX and Matrigel surfaces after long-term culture of human iPSCs

Summary

Human pluripotent stem cells (hPSC), including induced pluripotent stem cells (hiPSC), are a valuable resource for research and cell therapy applications. Successful culture of these cells in vitro requires a cell culture environment that effectively mimics the complexity of the natural extracellular matrix (ECM) while preserving the integrity of the hPSC cultures in terms of pluripotency and functionality.

A common matrix used for hPSC culture is Matrigel®, a basement membrane component derived from mouse EHS tumors. Though widely used since the first publication of mTeSR® media in 2006, Matrigel is far from an ideal substrate, as it is animal-derived, undefined, difficult to handle, and is highly inconsistent in both composition and concentration.

An ideal hPSC culture environment is suitable for both fundamental research and downstream cell therapy applications, with high consistency lot-to-lot and reliable performance over time. myMATRIX™ iPSC pre-coated plates provide a chemically defined ECM substrate that produces high-quality hPSC cultures and reproducible experimental results. myMATRIX iPSC pre-coated plates are compatible with common hPSC culture media and protocols, including mTeSR media, and both clump and single-cell passaging techniques, offering an easy transition from Matrigel to a hPSC culture system that meets today's basic scientific standards.

The Spotlight

Research area: Maintenance culture, expansion scale-up, and cloning of human pluripotent stem cells

Cell type(s): hPSCs, hiPSCs, and hESCs

Experiment purpose: A demonstration of performance of myMATRIX iPSC pre-coated plates as a suitable or superior alternative to Matrigel. Standard hiPSC culture conditions and techniques were compared using Matrigel and myMATRIX iPSC to show that myMATRIX iPSC plates are interchangeable with common media (mTeSR1) and workflows (clump and single-cell passaging, cell expansion, freeze/thawing, and cloning).

Product Highlights

myMATRIX™ iPSC Pre-Coated Cultureware

- Novel, chemically defined, and xeno-free biomatrix
- Proven ability to maintain healthy, pluripotent stem cells in long-term culture
- Customizable options to match scale-up workflows

Experiment overview

Long-term culture of hiPSC lines

- 3 hiPSC lines from different sources were used in the study
- Cells were cultured using myMATRIX iPSC pre-coated plates in parallel with Matrigel for 20 passages (140+ days) using mTeSR1 media and ReLeSR™ for standard clump passaging
- Cells were expanded in 2 sets of 10 passages, freezing banks of cells in each condition at p10 and thawing to continue culture through p20+

Single-Cell attachment and clonogenicity

- Single cells from 3 hiPSC lines were seeded at low densities
- Cultures were evaluated for initial attachment at 24 hours
- Survival and proliferation with and without the addition of a ROCK inhibitor was assessed for each matrix at 96 hours

Summary of comparative experiments conducted

- Attachment and morphology of transferred hiPSC cultures
- Survival and attachment after freeze/thaw cycle
- Growth speed of hiPSC lines over long term culture
- Pluripotency marker expression assessed by mRNA transcript quantification and immunophenotype
- Karyotypic analysis of each cell line
- Tri-lineage differentiation of lines cultured over 100 days

Results

The long-term culture comparison (20 passages) showed that hiPSCs cultured on myMATRIX iPSC pre-coated plates maintained their characteristic morphology. Distinctive and typical hiPSC morphology (round, compact, well-defined colonies containing cells with a high nuclear-cytoplasmic ratio and prominent nucleoli) was maintained succesfully with the myMATRIX iPSC plates (Figure 1).

Human iPSC lines on myMATRIX iPSC plates proliferate reliably with a similar growth speed to cells cultured on Matrigel. As expected, each cell line exhibited slightly different morphology and levels of colony compaction in culture, which was seen similarly in both Matrigel and myMATRIX cultures. While immediately after passaging, hiPSCs cultured on myMATRIX iPSC plates were observed to be less compacted than on Matrigel, observed morphologies were similar as the cultures reached confluency prior to the next passage.

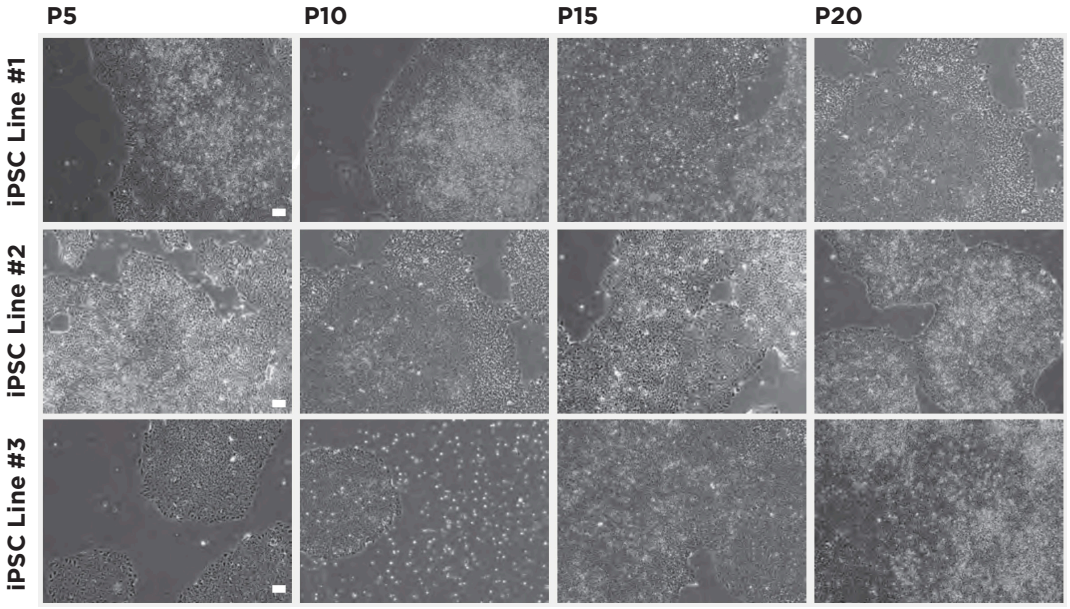

Figure 1. Representative images of hiPSCs on myMATRIX iPSC. hiPSC lines grown on myMATRIX iPSC were imaged after every 5 passages in culture using phase contrast imaging. The cells show an iPSC charateristic colony morphology with compacted individual cells that exhibit a high nuclear-to-cytoplasmic ratio.

Strong stability and marker expression of hiPSCs

Karyotype analysis was performed on all hiPSC lines at both passage 10 and 20 in culture, demonstrating chromosomal stability and genetic integrity of the cells after long-term culture. Pluripotency marker expression (both quantative mRNA expression by qPCR at p10 and immunofluorescence at p10 and p20 showed that cells maintained strong expression of pluripotency-associated genes (Figure 2).

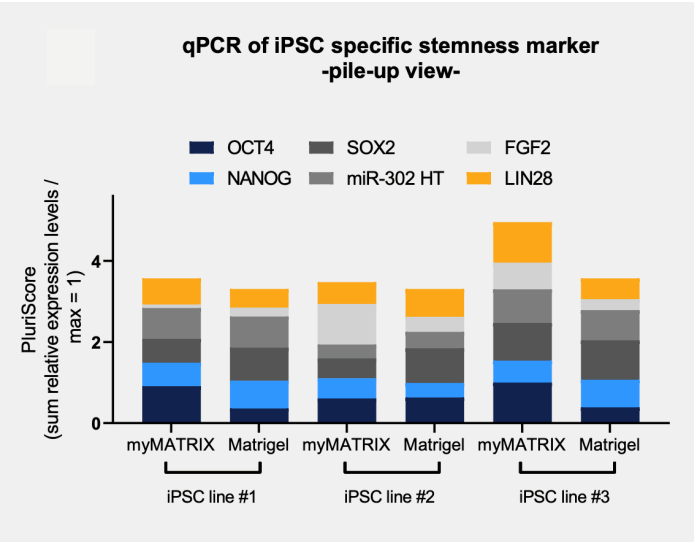

Figure 2. Quantitative qPCR of iPSCs cultured on myMATRIX iPSC and Matrigel for 10 passages. Human iPSCs were assessed for their expression of pluripotency-associated genes OCT4, Sox2, FGF2, Nanog, miR-302 HT and Lin28 using qPCR analysis after 10 passages on myMATRIX iPSC substrate. Data is expressed as Pluriscore, in which the sample with the highest expression was set to 1.

Efficient hPSC differentiation capacity

The functionality of the hiPSCs was assessed by demonstrating trilineage differentiation capability after 20 passages in culture. Key markers for endoderm, mesoderm, and ectoderm were uniformly expressed, showing efficient differentiation with no quantitative difference in capacity between myMATRIX iPSC plates and Matrigel (Figure 3).

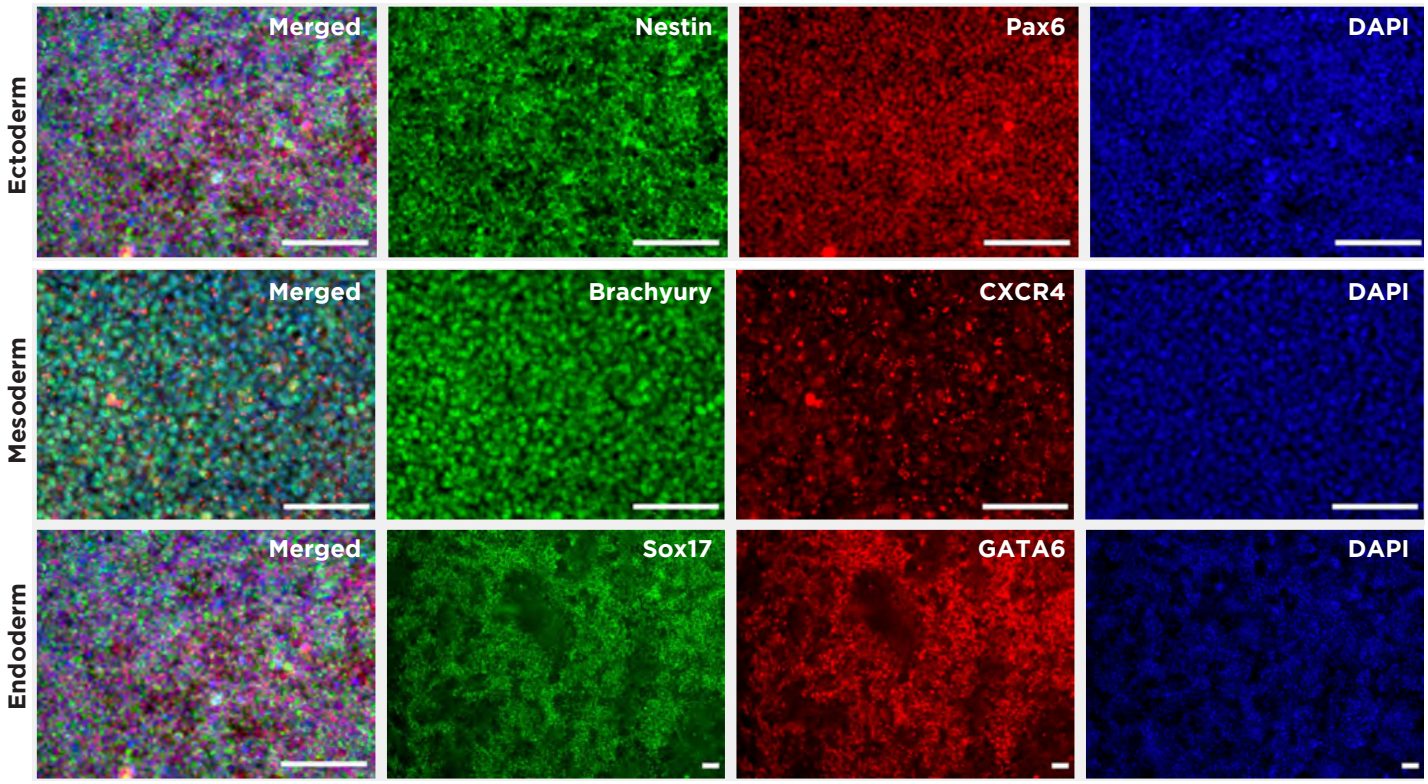

Figure 3. Immunostaining analysis of tri-lineage differentiation. Cells grown on myMATRIX iPSC were cultured for over 100 days and then differentiated towards the 3 embryonic germ layers. Shown are representative images of differentiated cells (20x). Images reflect an efficient differentiation of the iPSCs into all germ layers after long-term culture.

Summary

myMATRIX iPSC pre-coated plates are a reliable, xeno-free, and chemically defined alternative to Matrigel, with equal or superior attachment, proliferation, growth speed, and maintenance of iPSC stem-ness and differentiation potential, while maintaining characteristic morphology and genetic integrity over time.

Additional to these demonstrated features, myMATRIX iPSC pre-coated plates are an easy-to-use, highly consistent biomatrix that contributes to reliable and reproducible results over time, making this an excellent platform for both fundamental cell research and iPSC scale-up for cell therapy and regenerative medicine applications.

References

Segeletz, S. et al. 2021. White Paper No. 472. denovoMATRIX.

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