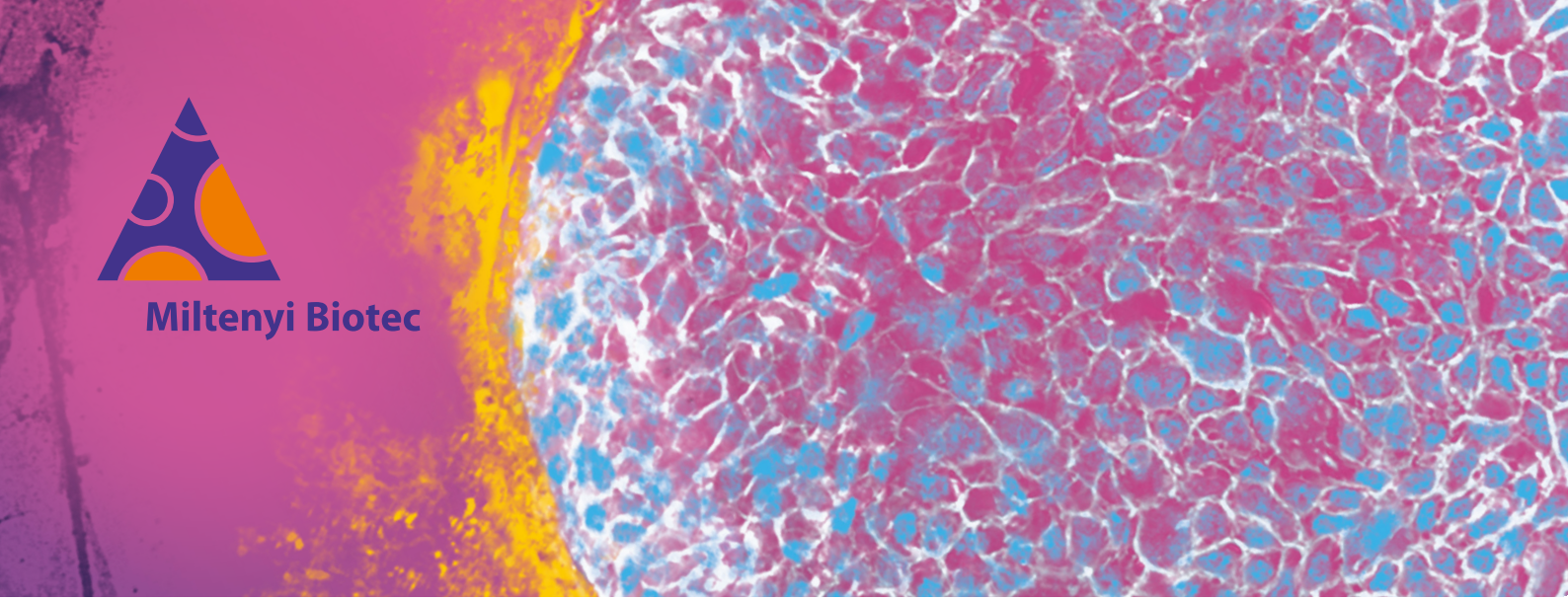


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A comparison of feeder-free cell culture media on stem cell viability and pluripotency

A review of TeSR and StemMACS™ media across multiple methodologies

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Background

Human pluripotent stem cells (hPSCs) possess the unique ability to self-renew or differentiate into various cell types based on the local environment. Cell culture is a critical part of stem cell research and the right media is critical for both maintaining healthy and actively proliferating cells and facilitating the ability to direct them into different lineages.

Since suboptimal media can affect pluripotency and differentiation potential of stem cells, it is crucial to choose the correct media before starting an experiment. In addition, the xenogeneic nature of embryonic feeder cells and commonly used media components is not compliant with current efforts to establish easily adaptable and clinically compatible protocols for the maintenance and differentiation of pluripotent stem cells.

In this application note, we successfully demonstrate that the xeno- and feeder-free StemMACS iPS-Brew XF media is a comparable alternative to the widely used mTeSR1 cell culture medium for human pluripotent stem cells (hPSCs). Specifically, we show how StemMACS iPS-Brew XF can maintain pluripotent morphologies over prolonged periods in culture without sacrificing developmental capacity.

Materials and Methods

Cell line generation and characterization
The hiPSC line was generated from normal/healthy primary dermal fibroblasts with non-integrating Sendai virus (SeV) vectors. The human embryonic stem cells (hESC) line was sourced from WiCell. Both cell lines were fully characterized and karyotyped to assess genomic stability prior to the study.

Cell culture

All cells were cultured in single lots of either StemMACS iPS-Brew XF (Miltenyi Biotec) or mTeSR™1 (STEMCELL Technologies) media on a single lot of Cultrex® Reduced Growth Factor Basement Membrane Extract (RGF BME, R&D Systems).

All passaging steps were conducted using a standard Versene-EDTA passaging protocol. The cells were only single-cell passaged for endpoint assays. When single-cell passaging was performed, 10µM of the ROCK inhibitor Y27632 was used overnight to help promote survival. Y27632 treatment was not used for routine passaging needs.

All cells used in this study were screened for Mycoplasma contamination using the MycoAlert™ Mycoplasma Detection Kit (Lonza Bioscience) prior to starting the study. Mycoplasma screening was also conducted throughout the study. No signs of Mycoplasma contamination were detected.

Microscopy

Imaging was performed on an IX51 inverted microscope (Olympus) equipped with an Infinity3 camera (Lumenera).

Flow cytometry

The manufacturer's recommendations were followed for all antibody staining processes. The FoxP3 buffer kit was used for antibodies with intracellular targets. Cells were fixed with 4% PFA after staining and stored at 4 °C prior to flow-cytometric analysis. Flow analysis was performed on a BD CANTO. Gates were established using isotype controls.

Trilineage directed differentiation

Directed differentiation was performed using the StemMACS Trilineage Differentiation Kit (Miltenyi Biotec). Briefly, single-cell suspensions were washed, trypsinized, and counted. Cells were plated at densities recommended by the protocol, followed by 10µM supplementation with the ROCK inhibitor Y27632. Cells were resuspended, then transferred to plates coated with Cultrex RGF BME (R&D Systems). Differentiation into the three germ layers proceeded as follows:

Mesoderm

- Day 1: the cultivation medium was replaced with StemMACS MesoDiff Medium I and incubated for 48 hours
- Days 4–6: the StemMACS MesoDiff Medium I was replaced daily with StemMACS MesoDiff Medium II

Endoderm

- Day 1: continued incubation with cultivation medium
- Days 2–6: the cells were washed daily with DPBS^{+} Ca2^{++} Mg2^{+} and incubated with StemMACS EndoDiff Medium

Ectoderm

- Days 1–6: the cultivation medium was changed daily to StemMACS Trilineage EctoDiff Medium.

On the seventh day, cells were analyzed using flow cytometry.

Results

hPSCs are easily transitioned into StemMACS iPS-Brew XF media and maintain strong morphologies over a prolonged period.

hiPSCs and hESCs were maintained in mTeSR1 feeder-free media, then transitioned to either StemMACS iPS-Brew XF or fresh mTeSR1 media. Morphologies (colonies with well-organized borders, small compact cells, and large nuclei) were assessed using 10× phase contrast imaging at passages 1 and 12. After the first passage, cells were easily transitioned to the StemMACS iPS-Brew XF with little to no differences in morphology. After 12 passages, hiPSCs and hESCs continued to show strong morphologies and no signs of stress or spontaneous differentiation in both the StemMACS iPS-Brew XF and mTeSR1 media (fig. 1). These results indicate that hPSCs transitioned to StemMACS iPS-Brew XF can maintain morphologies similar to those cultured in mTeSR1.

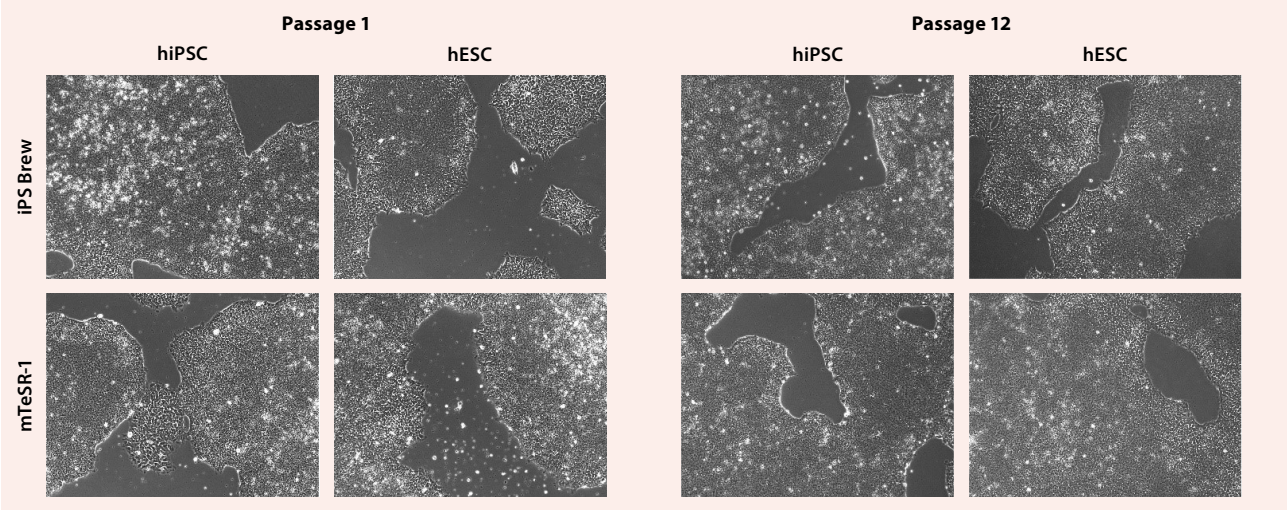


Figure 1: hPSCs are easily transitioned into the StemMACS iPS-Brew XF media conditions and maintain strong pluripotent morphologies over a prolonged period in culture. 10× phase contrast images of hiPSCs and hESCs cultured in the StemMACS iPS-Brew XF and mTeSR1 after 1 and 12 passages are displayed. After transitioning to the StemMACS iPS-Brew XF media conditions (from historic maintenance in mTeSR1), hiPSC and hESCs show strong morphologies (colonies with well-organized borders, small compact cells, and large nuclei) and no signs of stress or spontaneous differentiation. Additionally, after 12 passages in culture, hiPSCs and hESCs maintained in the StemMACS iPS-Brew XF continued to display strong morphologies consistent with hPSCs maintained in mTeSR1.

StemMACS iPS-Brew XF can maintain hPSCs in an undifferentiated state over a prolonged time in culture. To investigate the capacity of the StemMACS iPS-Brew XF to maintain cells in an undifferentiated state, hiPSCs and hESCs grown in the StemMACS iPS-Brew XF or mTeSR1 media for 12 passages were immunostained for TRA-1-81 and SSEA-4, traditional markers associated with undifferentiated human pluripotent stem cells.

In comparison, hiPSCs grown in the StemMACS iPS-Brew XF or mTeSR1 had similar numbers of double-positive cells (88.0% for StemMACS iPS-Brew XF vs. 86.4% for mTeSR1, fig. 2). Similarly, hESCs grown in the StemMACS iPS-Brew XF or mTeSR1 had similar numbers of double-positive cells (98.9% for StemMACS iPS-Brew XF vs. 95.8% for mTeSR1, fig. 2). These results indicate that hPSCs grown in StemMACS iPS-Brew XF maintain pluripotency similarly to those grown in mTeSR1.

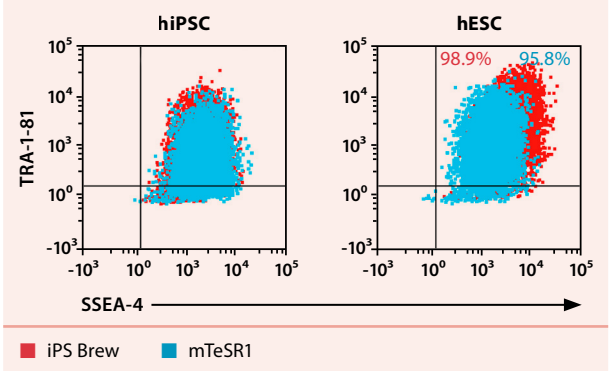


Figure 2: StemMACS iPS-Brew XF can maintain hPSCs in an undifferentiated state over a prolonged time in culture. The percentage of TRA-1-81 and SSEA-4 (traditional markers associated with undifferentiated hPSCs) double positive cells after 12 passages in StemMACS iPS-Brew XF and mTeSR1 media conditions is displayed. hESCs and hiPSCs cultured in StemMACS iPS-Brew XF maintain similar levels of TRA-1-81 and SSEA expression as compared to those cultured in mTeSR1. Cells cultured in the StemMACS iPS-Brew XF maintained high expression levels of both markers through a prolonged time in culture. Gates were generated from isotype controls.

hPSCs cultured in StemMACS iPS-Brew XF over prolonged periods can be efficiently differentiated towards endodermal, mesodermal, and ectodermal lineages. After 12 passages in either the StemMACS iPS-Brew XF or mTeSR1 media, hiPSCs and hESCs were subjected to directed differentiation using the StemMACS Trilineage Differentiation Kit followed by FACS staining for markers of the three germ layers: endoderm (CXCR4 and FoxA2), mesoderm (CD144 and CD140b), and ectoderm (PSA-NCAM and PAX6). Although some line-to-line variation existed, hiPSCs and hESCs cultured in either the StemMACS iPS-Brew XF or mTeSR1 were able to differentiate into all three germ layers with similar efficiencies (fig. 3). These results indicate that hPSCs grown in StemMACS iPS-Brew XF over prolonged culture periods can be differentiated into each of the three germ layers similarly to those grown in mTeSR1.

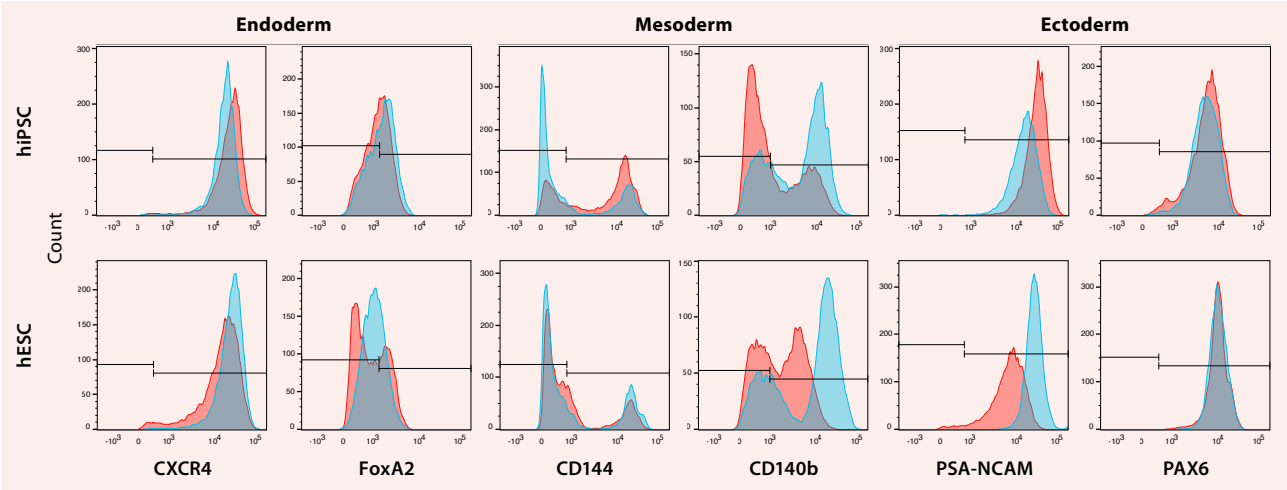


Figure 3: hiPSCs cultured in StemMACS iPS-Brew over prolonged periods can efficiently differentiate towards endodermal, mesodermal, and ectodermal lineages. After 12 passages in the StemMACS iPS-Brew and mTeSR1 media conditions, the developmental capacity of hiPSCs and hESCs was assessed through directed differentiations using the Miltenyi Trilineage Kit and FACS staining for associated markers of the respective germ layers. While some line-to-line variation exists in regard to the abundance of positively stained cells and expression/staining intensity, hiPSCs and hESCs cultured in the StemMACS iPS-Brew were able to differentiate to all three germ layers with similar efficiencies as cells cultured in mTeSR1. Gates were established from isotype controls.

Conclusions

Prolonged growth of hiPSCs or hESCs cultured in either StemMACS iPS-Brew XF or mTeSR1 were shown to:

- Maintain strong pluripotent morphologies with no signs of stress or spontaneous differentiation
- Remain in an undifferentiated state (shown for 12 passages)
- Differentiate towards endodermal, mesodermal, and ectodermal lineages

We conclude that the xeno- and feeder-free StemMACS iPS-Brew XF is a valid alternative to mTeSR1 for the culture of hiPSCs or hESCs.

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