A new generation cultivation medium that ensures optimal culture conditions in challenging PSC applications

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Introduction

Nowadays, human pluripotent stem cells (hPSCs) are often used in very challenging applications such as gene editing or cell sorting. Also after reprogramming, cells are put under stress by applying ultra-low density plating to generate new hPSC lines. The need of a stable and carefully composed medium environment becomes obvious to ensure survival and normal cell growth of hPSCs especially under stressful experimental conditions. Cells benefit from a constant nutrient and growth factor supply, a stable pH and low accumulation of degradation products, e.g. lactate or ammonium.

Here, we developed a xeno-free, new generation hPSC cultivation medium which contains stabilized FGF-2 to guarantee steady exposure levels of growth factors and therefore improves not only efficient maintenance and expansion of hPSCs but also the possibility of safely using flexible feeding strategies. When combined with an additional, optimized support supplement, it allows improved cell survival and stable cell growth of hPSCs after gene editing, cell sorting or reprogramming.

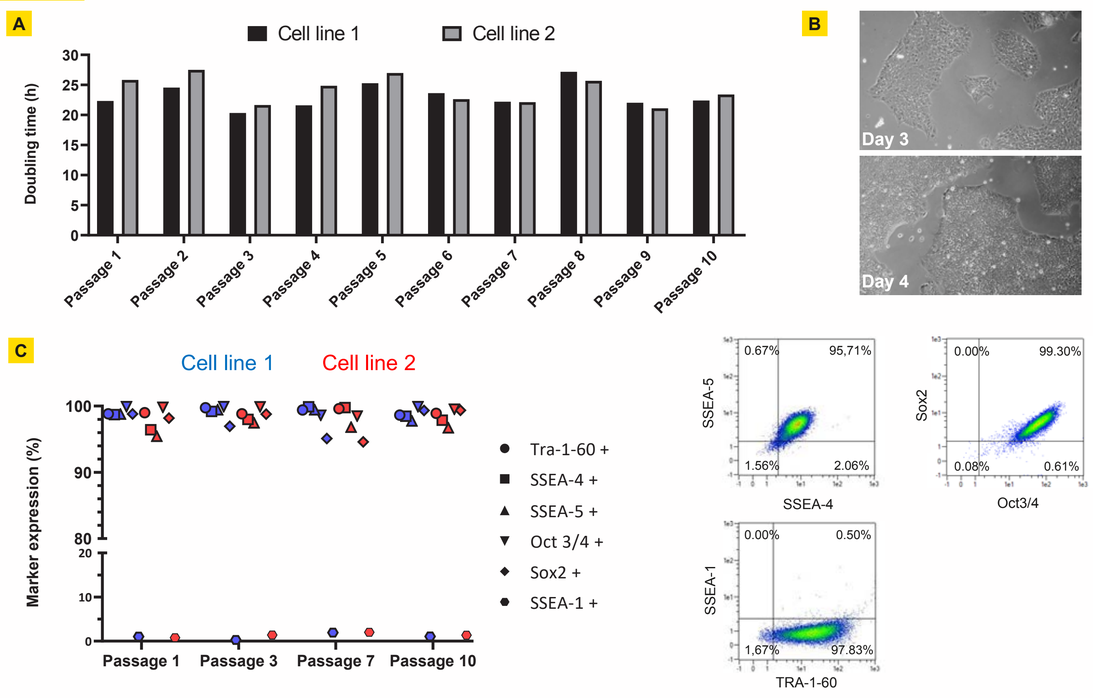
To evaluate the performance of the new generation medium, two hPSC lines were cultivated over 10 passages on Laminin521. Both cell lines maintained highly pluripotent and had a typical doubling time of 22-27 hours. They displayed a normal karyotype and could be efficiently differentiated into cells of all three germ layers.

To assess the potential of the additional support supplement, human fibroblasts were reprogrammed and resulting hPSCs sorted based on TRA-1-60/SSEA4 expression using the MACSQuant® Tyto® and plated into a 96-well plate. Later on, 6 PSC clones were chosen from the 96-well plate and further expanded using the new generation medium. The analyses at passage 4 and 5 revealed that all clones were highly pluripotent, displayed a normal karyotype and readily differentiated into mesoderm, ectoderm and endoderm.

Results

1 StemMACS™ PSC-Brew XF enables stable and efficient expansion of highly pluripotent stem cells

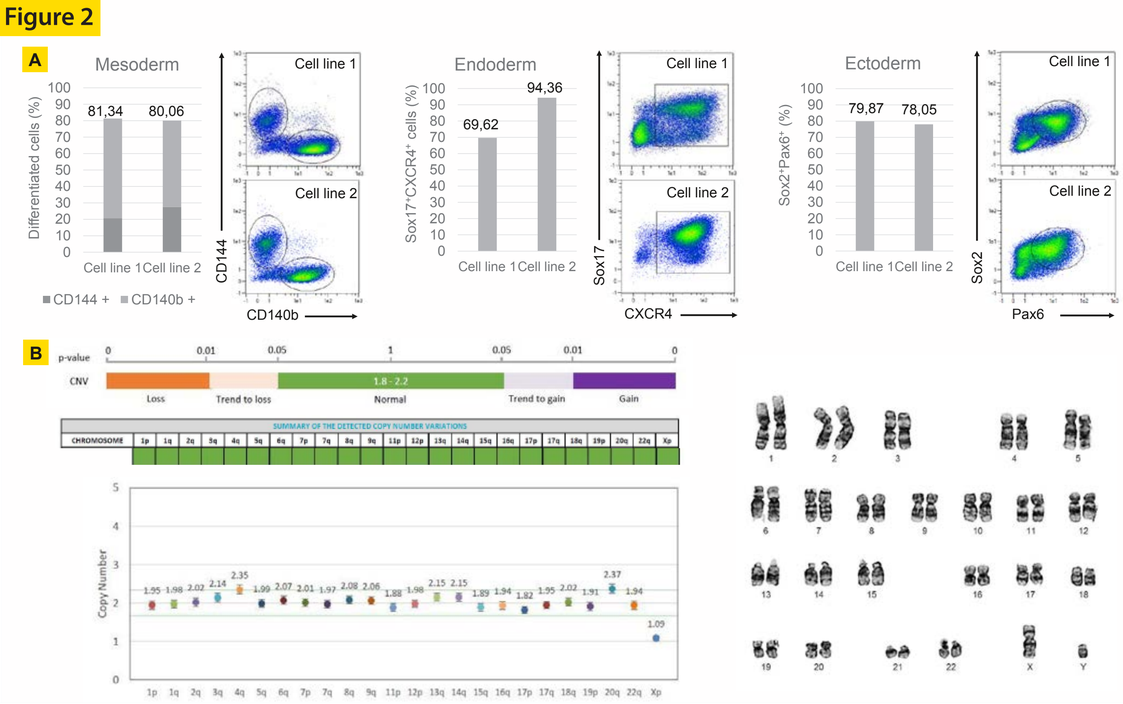
Figure 1



In order to evaluate the performance of StemMACS™ PSC-Brew XF, human we cultivated two hPSC lines over 10 passages as single-cells on Laminin521. Both cell lines had a typical doubling time of 22-27 hours and showed a homogeneous morphology during cultivation (A, B cell line 1). To confirm the quality of the cells, marker expression was checked at different passages using flow cytometry. Both cell lines displayed a high and persistent expression of pluripotency markers during the entire cultivation time: TRA-1-60 (98,6 – 99,7%), SSEA-4 (96,4 – 99,9%), SSEA-5 (95,5 – 99,4%), Oct-3/4 (98,5 – 99,9%) and Sox2 (94,5 – 99,4%) and showed only low expression of SSEA-1 (0,3 – 2%) (C, dot plots cell line 1 passage 10).

2 Cells cultivated in StemMACS PSC-Brew XF can be efficiently differentiated and retain a normal genotype

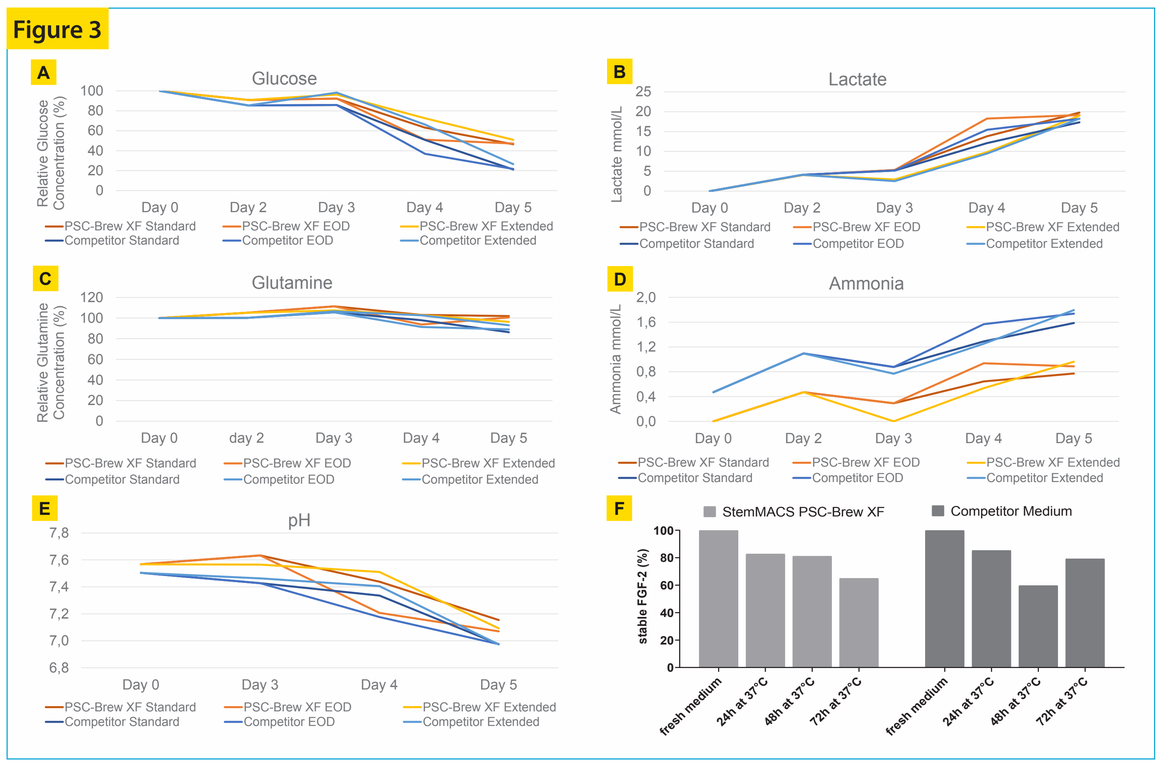
Figure 2



To determine the differentiation capability of both cell lines after 10 passages of cultivation in StemMACS PSC-Brew XF, cells were differentiated using the StemMACS™ Trilineage Differentiation Kit. Quantitative flow cytometry analysis confirmed their ability to differentiate into CD140b+ vascular smooth muscle cells or CD144+ endothelial precursors (mesoderm) (overall differentiation efficiency: cell line 1 81,34%, cell line 2 80,06%), Sox17+CXCR4+ definitive endoderm cells (endoderm) (cell line 1 69,62%, cell line 2 94,36%) and Sox2+Pax6+ neuroectoderm cells (ectoderm) (cell line 1 79,87%, cell line 2 78,05%) (A). The genomic stability of the cells was analyzed by karyotyping and in more detail by iCS-digital™ PSC 24-probes kit (Stem Genomics), a digital PCR which can detect more than 90% of recurrent numeric aberrations in hPSCs. Both methods revealed a stable genotype for both cell lines after 10 passages of cultivation (B, cell line 1, representative picture).

3 StemMACS PSC-Brew XF provides a carefully composed medium environment with constant growth factor supply

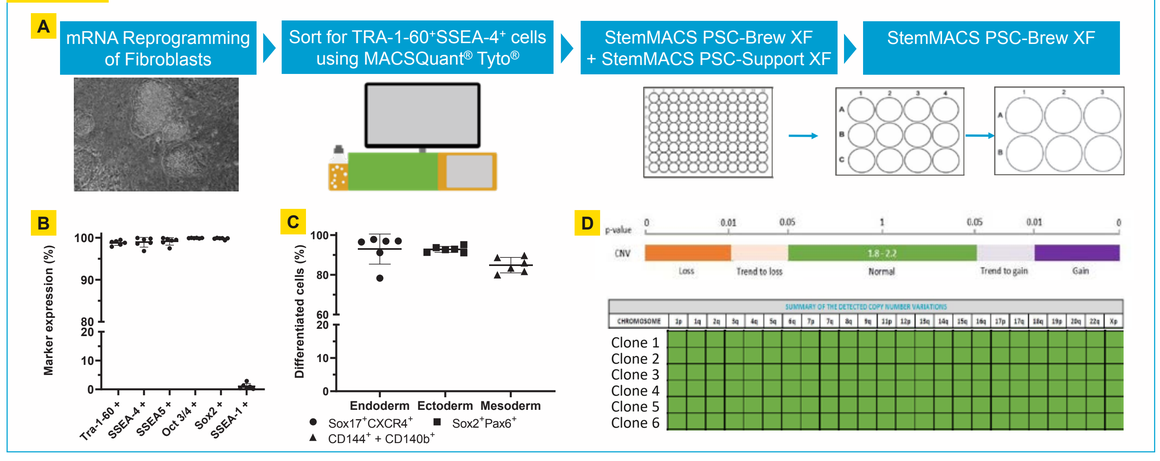
Figure 3



To assess the potential of the medium in terms of nutrient and growth factor supply as well as for accumulation of degradation products, we cultivated cell line 1 for 5 days in StemMACS PSC-Brew XF and a competitor medium, and analyzed the medium supernatant between day 2 and day 5 of cultivation. Additionally, different feeding strategies were applied: every day media change (Standard, 2ml/well), every other day media change (EOD, 2ml/well) and an extended media change using only one medium change at day 2 (Extended, 4ml/well). Under all three feeding regimes StemMACS PSC-Brew XF maintained more steady glucose levels (A), while exhibiting comparably increasing lactate levels (B). Glutamine levels remained comparably stable for both media (C), while with StemMACS PSC-Brew XF the accumulation of toxic ammonia was significantly reduced (D). Both media exhibited comparable stability with regard to pH (E) and FGF-2 concentration (F).

4 StemMACS PSC-Brew XF in combination with StemMACS PSC-Support XF facilitates efficient cell survival under stressful culture conditions

Figure 4



To demonstrate the synergistic effect of StemMACS PSC-Brew XF and the additional supplement StemMACS™ PSC-Support XF, human in challenging culture conditions, we reprogrammed human fibroblasts and sorted for TRA-1-60+SSEA4+ cells using the MACSQuant Tyto. Sorted cells were plated into a 96-well plate and cultivated for 4 days using StemMACS PSC-Brew XF supplemented with StemMACS PSC-Support XF. Then, cells were maintained in StemMACS PSC-Brew XF only. After 10 days of culture, 6 clones were chosen and further expanded (A). Quality control of all clones at passage 4 showed a high expression of the pluripotency marker TRA-1-60, SSEA4, SSEA5, Oct3/4 and Sox2 with >96% and only low expression of the differentiation marker SSEA-1 (<3%) (B). Additionally, clones were checked for their differentiation potential and genomic stability at passage 5. All clones could successfully differentiate into endoderm: 78 – 98% Sox17+CXCR4+ definitive endoderm cells, ectoderm: 91 – 95% Sox2+Pax6+ neuroectoderm cells, and mesoderm: 80 – 90% overall differentiation efficiency of CD140b+ vascular smooth muscle cells or CD144+ endothelial precursors (C), no genetic abnormalities could be detected by using iCS-digital PSC 24-probes kit (D).

Conclusion

Here we developed a xeno-free, new generation PSC cultivation medium which:

- supports stable and efficient expansion of highly pluripotent stem cells,
- provides a constant nutrient and growth factor supply, a stable pH and low accumulation of toxic degradation products,
- when combined with StemMACS PSC-Support XF, enables cell survival and stable cell growth of PSCs under stressful culture conditions.