Clinical grade culture medium for expansion, large-scale culture and genome engineering applications of human pluripotent stem cells

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Introduction

Lack of robust methods for clinical expansion of human pluripotent stem (hPS) cells still hampers their clinical use. Here we report the development of a culture system for expansion of hPS cells that is totally free from human- or animal-derived components, chemically defined, containing only raw materials of clinical quality with traceable production processes. Cells expanded in regular T-flasks in monolayer in this culture system maintain a high expression of stem cell markers and lack expression of differentiation markers up to 30 serial passages, display long-term self-renewal potential, retained stem cells characteristics and differentiation potential. The culture system further supports the expansion of an array of hPS cell lines using a very robust and straightforward single-cell based culture protocol[1,2]. Generation of clinically relevant quantities of hPS cells, ranging from 10^8 or beyond, is essential for their clinical use[3]. In addition to the regular T-flask expansion described above, the culture medium also support large-scale non-adherent expansion of hPS cells in suspension culture.

Genome engineering is a process that presents an additional challenge for hPS cells[4] and robust and pampering culture conditions is required in order to succeed. Here we show data on how this xeno-free, defined and feeder-free culture system support hPS cells also during the genome engineering process.

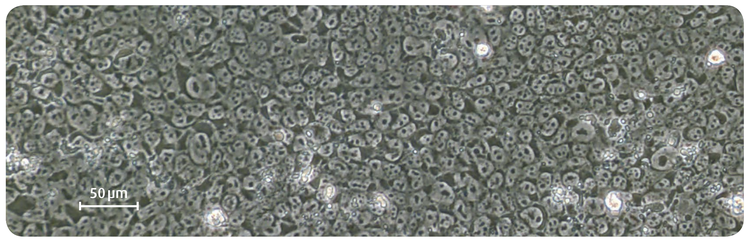


Figure 1: Morphology of cells cultured in DEF-clinical.
Morphology of hESC line SA121 expanded in the DEF-clinical culture system for 23 passages. The above picture is representative for the 6 hPSC lines tested (both hESCs and iPSCs), some expanded up to passage 30. No morphological signs of differentiation was seen and the cells grew as a homogenus monolayer culture.

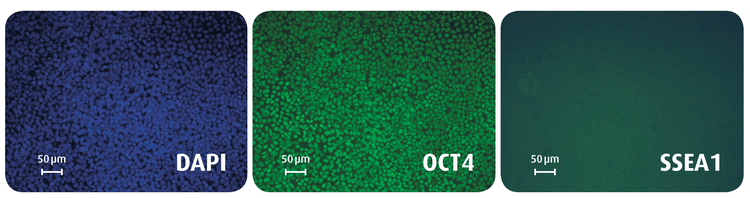


Figure 2: Immunocytochemistry of cells cultured in DEF-clinical
Immunocytochemistry analysis of hESC line SA121 cultured in the DEF-clinical culture system for 30 passages. The staining revealed high protein content of the hESC marker Oct4 and lack of expression of the early differentiation marker SSEA1. These stainings are representative for other hESC/iPSC lines tested.

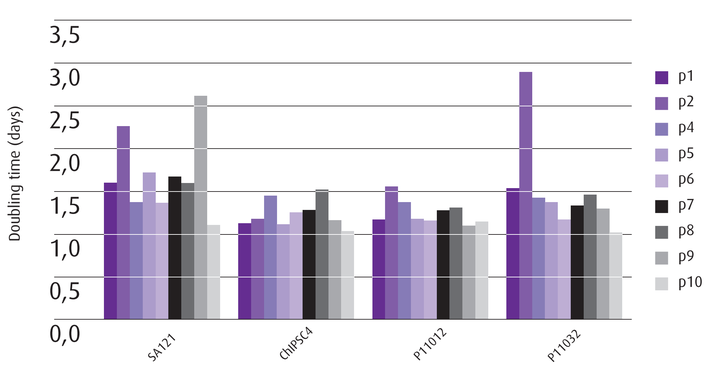
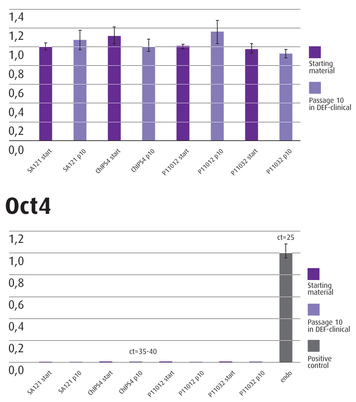


Figure 3: Proliferation of cells cultured in DEF-clinical
Proliferation rate of 4 different pluripotent cell lines (both hESCs and iPSCs) expanded in the DEF-clinical culture system up to passage 10. Proliferation displayed as the population doubling time in days. Cells were expanded every 3-4 days, at most passages demonstrating a population doubling time of 1-1,5 days.



Oct4 **MESP1** **SOX17**

Figure 4: Gene expression of cells cultured in DEF-clinical
Gene expression of the hESC marker Oct4 as well as the mesodermal marker MESP1 and endodermal marker SOX17. First the cells were expanded in a commercially available culture medium of non-clinical grade (green bars) and was then transferred to the DEF-clinical culture system and cells were then expanded for another 10 passages (red bars). hESCs differentaited into the mesodermal and ectodermal lineages were used as positive controls for MESP1 and SOX17 expression. These results are representative for other hESC/iPSC lines tested.

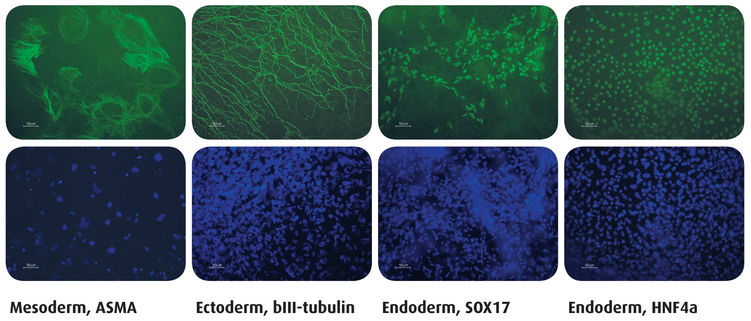


Figure 5: Differentiation potential of cells cultured in DEF-clinical
Pluripotent stem cells expanded in the DEF-XF culture system for 10 passages where then analyzed for their potential to differentiate into the three germ layers using embryoid body formation. As can be seen, the cells have the potential to differentiate into tissues representative for all three germ layers.

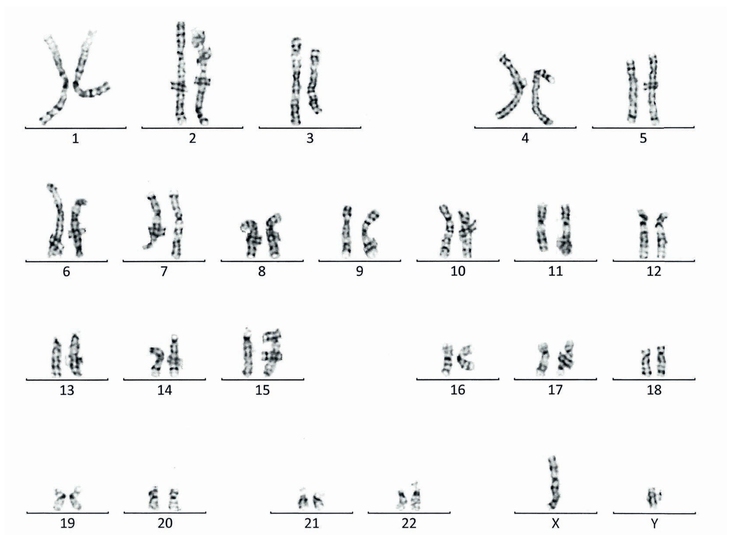


Figure 6: Karyotyping of cells cultured in DEF-clinical
20 spreads from cells (cell line SA121) expanded in the DEF-clinical culture system for 15 and 30 passages where karyotyped to study genomic stability. The results showed that the cells displayed a normal diploid karyotype.

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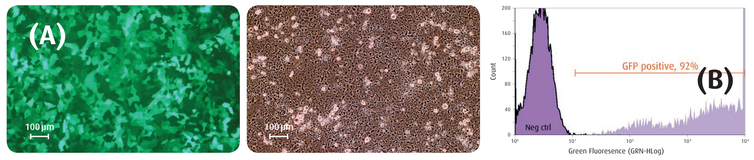

Figure 7: Genome engineering in the DEF-clinical culture system
(A) GFP expression by fluorescence microscopy 48 h post transfection. Magnification 10 x. The human ES cell line SA121 was cultured in the DEF-clinical culture system for 12 passages. Transfection with an EF-1alpha driven eGFP construct was done utilizing the electroporation based technology. GFP expression was analyzed 48 h post transfection by flow cytometry and gave over 90 % GFP expressing cells. (B) Quantification of GFP expression 48 h post transfection by flow cytometry.

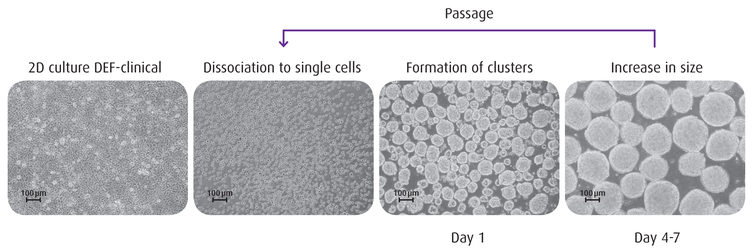

Figure 8: Expansion of cells cultured in suspension in DEF-clinical
In order to find a system that would work for expansion of a larger number of cells for clinical use, suspension culture as spheroids of pluripotent stem cells were optimized. Cells cultured using this type of method in the DEF-clinical culture medium nicely formed homogenous, round spheroids and displayed good proliferation rate.

Conclusions

DEF-clinical culture system is a defined and feeder free medium of cGMP grade, free from human- and animal-derived components. Characterization of hPS cells that have been cultured in the DEF-clinical culture system express the expected stem cell markers, remains diploid normal and the cells can differentiate into cell types from the three germ layers. Further, the system allows for robust and scalable production of hPS cells for clinical use. As the culture system also supports hPS cells after transfections it also allows for genome engineering on a cGMP level. This system can thus facilitate the use of hPS cells for research as well as in large-scale clinical applications.

References

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