Expansion of 3D human induced pluripotent stem cell aggregates in bioreactors with a clinical grade culture medium

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Abstract

Human induced pluripotent stem cells (hiPSCs) are attractive tools for drug screening and disease modeling as well as promising candidates for cell therapy applications. Here we present the development of a defined, feeder-free medium, without human- or animal-derived components. hiPSCs that are cultured in this medium for an extended period of time express expected stem cell markers, remain diploid, and can differentiate into cell types from the three germ layers. Using this complete, clinical-grade culture medium, eight different hiPSC lines that were expanded as a 2D monolayer (2D culture) maintain high expression of pluripotent stem cell markers and lack any expression of differentiation markers over the 12–20 passages tested. In addition, no karyotype abnormalities were reported for any of the tested cell lines.

In order to generate clinically relevant quantities of hiPSCs—10^9 and beyond—it is essential to develop efficient, robust 3D suspension cultures maintaining the same stability as 2D monolayer cultures. Previous reports in the literature of suspension cultures have typically described a reduced growth rate compared to monolayer cultures with a final cell concentration of 1–2 million cells per milliliter. We demonstrate that our culture system supports large-scale, 3D, non-adherent expansion of hiPSCs in suspension culture in a perfusion bioreactor. Furthermore, by optimizing perfusion rates and dissolved oxygen levels, we were able to expand hiPSCs 1,100-fold within 3 passages over 11 days to a final concentration of 5 million cells per milliliter using our 3D suspension culture system. In summary, our clinical-grade culture system allows for efficient, robust, and scalable production of hiPSCs, thus facilitating the use of hiPSCs for research and large-scale 3D suspension clinical applications.

1 Stable proliferation rates and long-term preservation of genomic stability

2 Cells maintain their pluripotency without differentiation

3 Cells maintain their differentiation potential into the three germ layers

4 Expansion workflow for 3D suspension culture

5 Comparison of dissolved oxygen concentrations for optimized cell growth in 3D culture

6 Comparison of dissolved oxygen concentrations for optimized pluripotency in 3D culture

7 Scalable expansion of hiPSCs in bioreactors under optimal O2 conditions

8 Scalable expansion of hiPSCs in bioreactors under optimal culturing conditions maintains pluripotency after multiple passages

9 After 3D scale-up, hiPSCs maintain the capacity to differentiate into beta cells

Conclusions

• Cellartis DEF-CS xeno-free medium is defined and completely free of human- and animal-derived components.
  • This culture system is optimized for feeder-free culturing of hiPSCs in 2D-monolayer or 3D-suspension-culture formats.
  • hiPSCs cultured in this system express the expected stem cell markers, remain diploid, and differentiate into cell types from the three germ layers.
  • This culture system has been optimized for the oxygen concentration that facilitates the highest levels of pluripotency in 3D formats.
  • The system allows for robust and scalable hiPSC production in a perfusion bioreactor, facilitating the use of hiPSCs for research and large-scale clinical applications.

Cells grown in 3D suspension can efficiently differentiate into beta cells, although cells only expand through the early progenitor phase of differentiation.

References


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