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Animal Component-free induced Pluripotent Stem Cell Lines

Manufacturing of GMP-Grade induced Pluripotent Stem Cells

Highlights

• Animal-component/Xeno-free process

• Non-invasive cell sourcing

• Virus-free cell reprogramming

• Scalable cultivation processes and -development

• GMP-grade cell banking (soon)

Background and Introduction

Stem cells and stem cell-derived products (extracellular vesicles) provide an outstanding therapeutic perspective for multiple diseases[1]. Therefore, we established lab-scale cell cultivation procedures that are currently optimized and developed into scalable manufacturing processes[2]. Phoenestra develops technology platforms to meet the rising demand of the gene and cell therapy field.

Methods

We established procedures for animal component-free (xeno-free) induced pluripotent stem cell (iPSC) line generation from patient-derived urine donations within 10 weeks. This non-invasive cell sourcing enables easy sampling from non-healthy patients. Cells are isolated using aseptic conditions and expanded for reprogramming (minimal required cell doublings/age). Reprogramming with episomal vector-based expression of six factors (Oct4, Sox2, Lin28, Klf4 and L-Myc) together with the expression of Mp53DD and EBNA1 results in high reprogramming efficiency and prevents toxicity and impurity issues. Several clones are selected for further expansion and testing. Standard cell line quality control includes marker

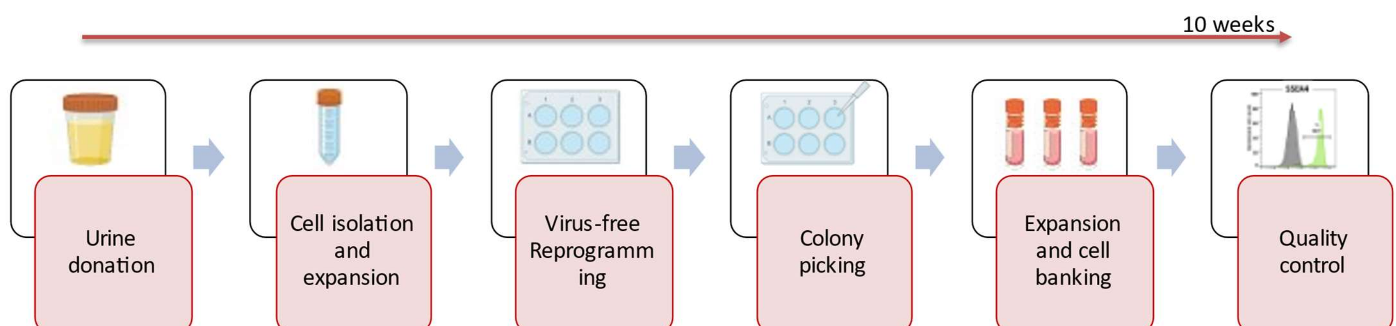

Figure 1: Cell Isolation and Reprogramming for induced pluripotent stem cells (iPSCs)

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expression, differentiation into the three germ layers (endo-, meso-, ectoderm), karyotyping and microbial/virus testing. After reprogramming, cell banking is done at passage 10 for optimal stability.

At lab scale, iPSCs are expanded in cell culture flasks (2D, adherent) or shake flasks (3D, suspended). Spheroids that are formed in suspension stabilize pluripotency and enhance growth performance of the iPSCs.

Manufacturing of high-quality iPSC lots is performed in stirred tank bioreactors (3D) with pH, dissolved oxygen (DO) and temperature control. Metabolites, growth performance (cell counting after dissociation) and cell viability are monitored offline.

Figure 2: Stirred tank bioreactors used for process development (Eppendorf DASbox)

The manufacturing process is controlled, reproducible, scalable and applies serum-free materials. Xeno-free iPSC manufacturing processes are under development.

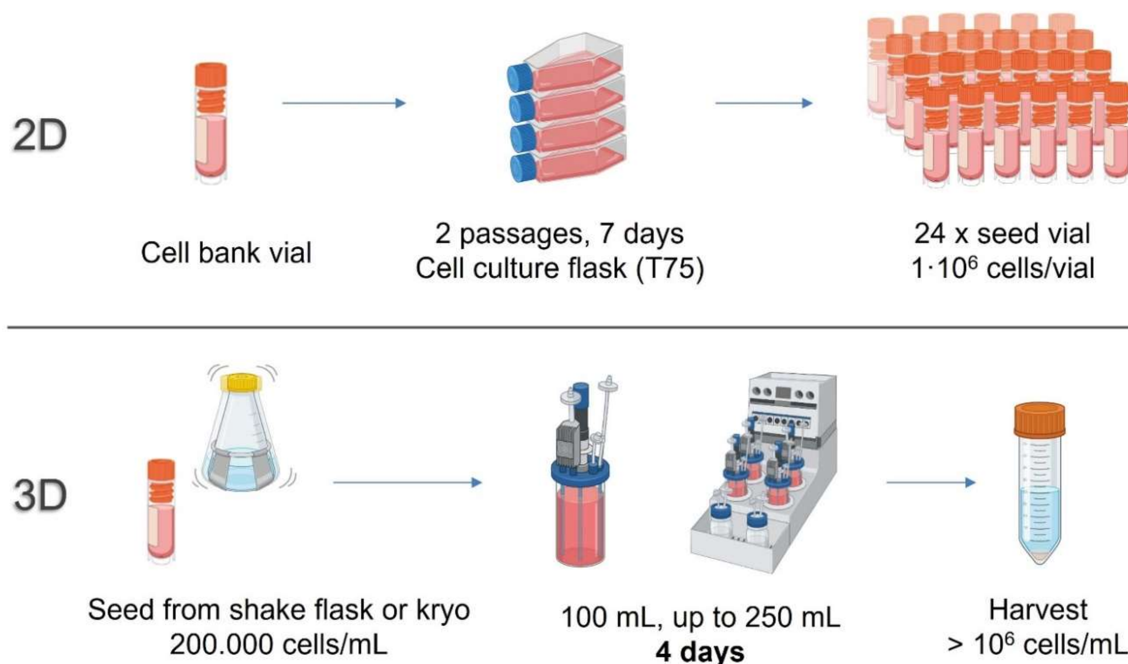

Figure 3: Stem Cell Proliferation in 2D and 3D

Results

Conventional 2D cultivation of iPSCs is done using xeno-free medium and coating. Cells are passaged twice a week (single cells or aggregates) with a weekend-free feeding strategy. Growth performance is stable with a growth rate (μ) of 0.45-0.65 d^{-1} (dependent on cell line, cultivation media etc.) and pluripotency was confirmed.

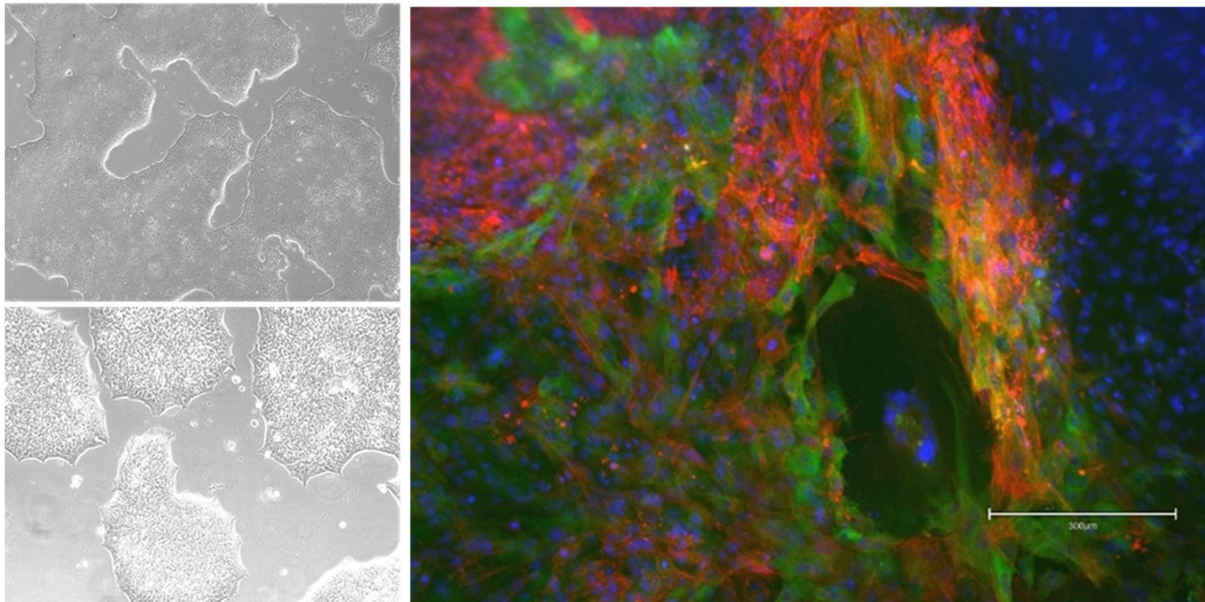

Figure 3: Spontaneous differentiation via embryoid body formation. Mesoderm and ectoderm staining with α-actin Alexo Fluor 546 and β3-tubulin FITC; counterstaining with DAPI

Small scale cultivations produce highly viable iPSCs, with up to 30 million cells per shake flask. Compared to adherent culture, the volumetric cell yield of spheroid culture in shake flask is more than 3-fold. The current iPSC bioreactor process delivers up to 2 billion ($2x10^9$) cells per Liter of culture within 4 days. Pluripotency of the cells was confirmed by flow cytometry analysis.

Perspective

We have successfully established key elements for supplies of clinical grade iPS cell lines:

- Fully compliant sourcing of donor material (e.g., urine samples)
- Xeno-free, fully documented reprogramming of urine cells to iPSCs
- Cloning and stability testing on iPSC clones
- Cell banking process
- Scalable expansion of iPSCs to significant cell numbers (>10^9 cells / mL)
- Key quality control methods
- GMP manufacturing (coming soon)

The platform technology is currently expanded towards:

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HLA-gene edited iPSC (Phoenestra IP)

Combining iPS cell expansion with differentiation processes

Vesicle processing and characterization

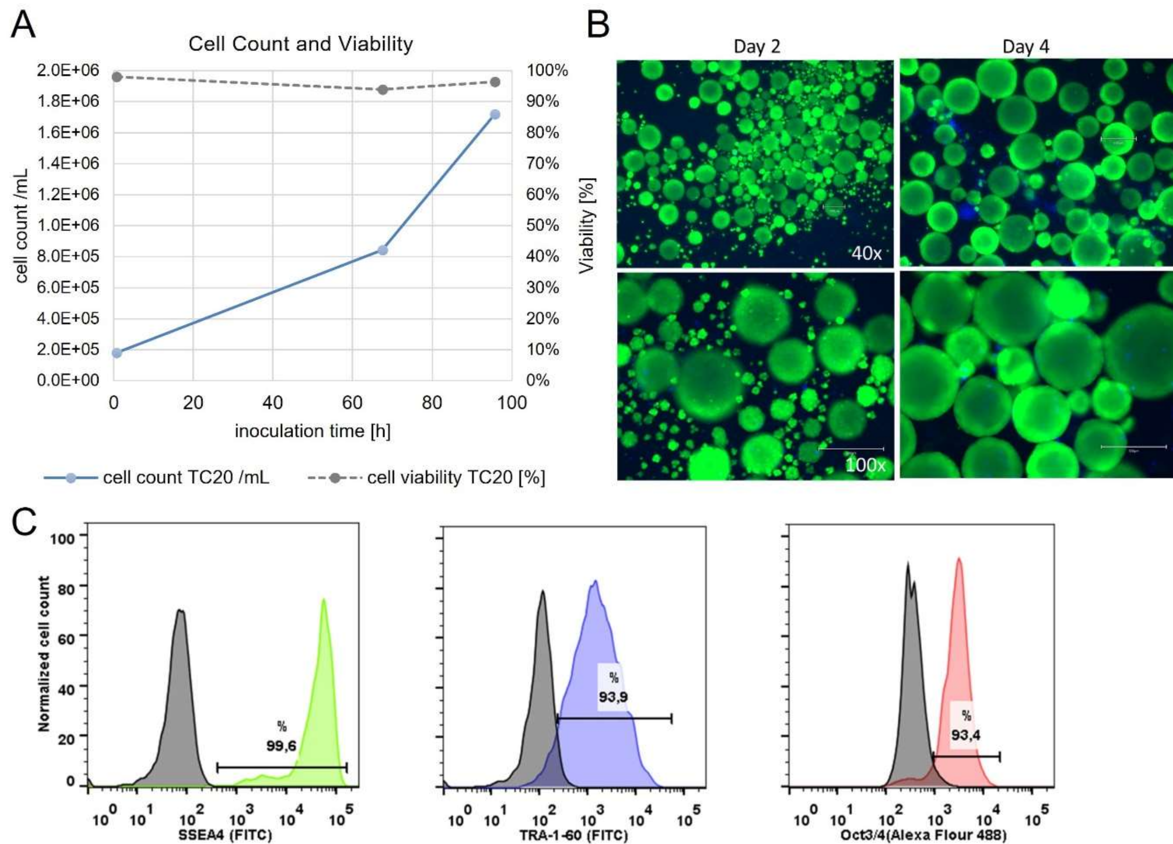

Figure 5: (A) Cell density and viability of iPSCs in bioreactor cultivation. (B) Microscopic images of iPSC spheroids from bioreactor cultivation (Calcein-AM live cell staining). (C) Flow Cytometry Analysis confirmed comparable stem cell marker expression of iPSCs grown in a stirred tank bioreactor under serum-free conditions.

[1] A.E. El-Kadiry, M. Rafei, R. Shammaa, Front Med (Lausanne) 2021, 8:756029. DOI:10.3389/fmed.2021.756029

[2] C. Kropp, H. Kempf, C. Halloin, Stem Cells Transl Med. 2016, 5(10):1289-1301. DOI: 10.5966/sctm.2015-0253