BIOTHRUST

Advancing human iPSC cultivation processes in bionic bioreactors

Authors: Patrick Bongartz[1], Yasemin van Heuvel[1], Misha Teale[2], Daniel Terheyden-Keighley[3]

[1]BioThrust GmbH, Pauwelsstraße 17, 52074 Aachen, Germany; [2]ZHAW Life Sciences and Facility Management, Centre for Cell Cultivation Techniques, Tissue Engineering, and Medical Biology, Grüentalstrasse 14, 8820 Wädenswil, Switzerland.
[3]Catalent Inc., Berghausener Str. 98, 40764 Langenfeld (Rheinland), Germany

Development & Technology

Abstract

These proof-of-concept (POC) studies present preliminary results in developing scalable cultivation processes for human iPSCs and iPSC-derived immune cells. A key innovation of BioThrust's novel bionic bioreactor, the Comfy*Cell*, equipped with a unique Membrane Stirrer (MemStir), is that this stirrer enables low shear conditions and efficient gas transfer through diffusion, eliminating gas bubbles and foam formation. These optimized conditions support the scalable production of shear-sensitive stem and immune cells in a 3D environment that more closely mimics physiological conditions than conventional STRs. A major challenge in scaling of hiPSCs is their sensitivity to shear stress (τ) introduced through bioreactor agitation and bubble rupture, which has been shown to impact cell growth and quality. Accordingly, these studies showcase preliminary results in developing scalable human iPSC and iPSC-derived immune cell cultivation processes in a bionic bioreactor setup at 250 mL and 2 L scales, while preserving intrinsic cell characteristics.

Materials & Methods

Introduction

3D production systems using various cell lines need optimal cultivation conditions specifically adapted to their needs. Conventional stirred tank bioreactors (STRs) are among the most widely used systems due to their versatility and broad applicability, but struggle with high shear, insufficient gas transfer, and/or foam formation. To overcome these challenges, BioThrust developed a novel membrane stirrer (Mem*Stir*), which is used for both mixing and aeration of STRs. Aeration is provided through bubble-free diffusion at the membrane-liquid interphase. The elimination of gas-bubbles reduces shear stress, while ensuring an ample supply of oxygen and high volumetric mass transfer rates ($k_L a$), alongside the elimination of foam [1]. In the following, properties and performance of the Mem*Stir* are displayed.

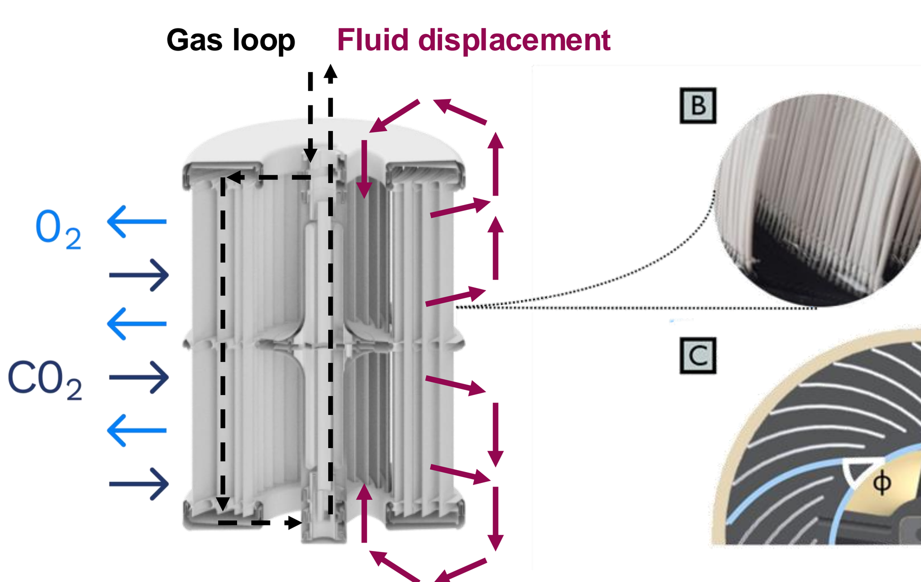

Figure 1 Schematic images of the Mem*Stir*. **A.** Side cross section portraying the open-loop gas flow (pink, blue and black arrows) and the fluid displacement (dark blue arrows). **B.** Close-up of Mem*Stir* blade consisting of fixed hollow-fibers. **C.** Top-down perspective of a Mem*Stir* section, which shows the placement angle ϕ of the vertical membranes.

The Mem*Stir*

The Mem*Stir* consists of multiple 3D-printed medical-grade parts that are assembled to a hollow module housing (see Fig. 1). Vertically inserted dense hollow-fiber membranes are connected through the hollow space, enabling an open-loop tangential gas flow (see Fig. 1A, B). Prior to application, the stirrer is mounted onto a modified stirrer shaft allowing module rotation during aeration. In this manner, a radial flow profile is generated, displacing the fluid towards the reactor wall and subsequently drawing it in from the intermediate space (see Fig. 1A). Gas is introduced into the system through membrane diffusion, preventing bubble and foam formation while achieving higher $k_L a$ values (> 20 h^{-1}) compared to conventional STRs (see Fig. 3). Due to the cyclone shape of the Mem*Stir* (see Fig. 1C), module stirring generates lower local shear than conventional stirrers, with a maximum shear rate of 400 s^{-1} at the membrane tips corresponding to an average shear rate of only 8.9 s^{-1} (see Fig. 4) [2]. Accordingly, this design was optimized to scale linearly from 250 mL to 200 L and beyond (see Fig. 2) [3].

Scales

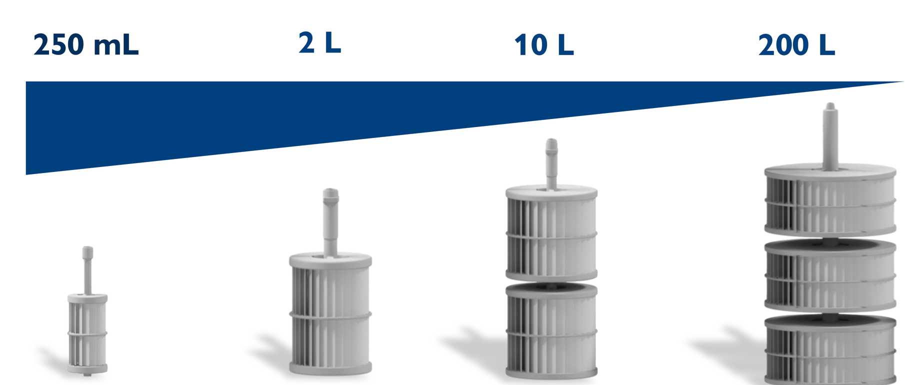

Figure 2 Current Mem*Stir* sizes for different process volumes.

Oxygen

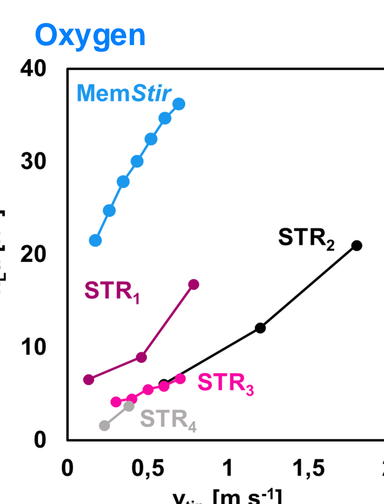

Figure 3 Determined $k_L a$ values for laboratory bioreactors (1.5– 3L) using the sulfite oxidation method under physiological conditions (37°C, pH 7, 0.1 vvm, 50–650 rpm).

Shear rates

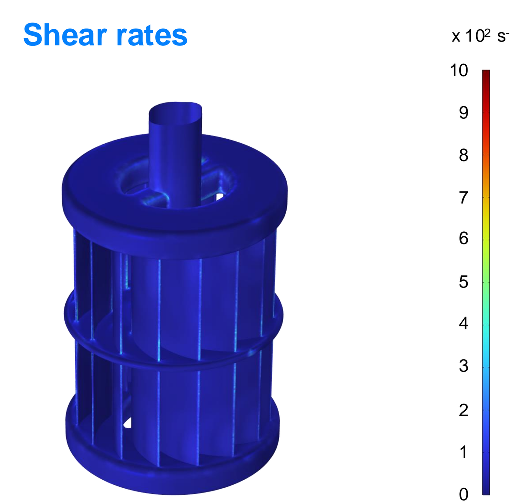

Figure 4 CFD-simulation of local Mem*Stir* shear rates given in s^{-1} when operated at 60 rpm (or a tip speed of 0.13 m s^{-1}) and a working volume of 300 mL in the Comfy*Cell*.

1. High $k_L a$ values suitable for a wide variety of cultivation conditions.
2. Superior oxygen supply of up to 180 mmol L^{-1} h^{-1}.
3. Designed to achieve near-absolute mixing homogeneity.
4. Shear rates can significantly be reduced!
5. No foam formation!

Application Studies

Deep-dive: hiPSC expansion in Comfy*Cell*$_{mini}$

Misha Teale, Yasemin van Heuvel, Moritz Meyer, Patrick Bongartz, Regine Eibl
ZHAW Life Sciences und Facility Management, Centre for Cell Cultivation Techniques, Tissue Engineering, and Medical Biology, Grüentalstrasse 14, 8820 Wädenswil, Switzerland

Introduction

This application note presents a first of its kind benchmark study showcasing preliminary results in the development of scalable human iPSC cultivation processes on microcarriers in a bionic bioreactor setup reaching more than 3.5 M viable iPSCs mL^{-1} at an exceptional fold expansion of 65. Phenotypic pluripotency markers were maintained and differentiation into all three cotyledons could be demonstrated following the final cell harvest.

The Comfy*Cell* proved its applicability in scaling dynamic stem cell processes using microcarriers and human iPSCs. These controlled conditions enable the scalable and physiologically relevant expansion of shear-sensitive stem cells in a three-dimensional (3D) culture system, supporting production demands for clinical applications beyond the lab or mL-scale.

1. A 65-fold expansion was reached in a first POC run at 60 rpm.
2. More than 3.5 M cells mL^{-1}, allowing 1 B hiPSCs to be produced in a single Comfy*Cell*!
3. All phonotypic identity marker & the full differentiation potential were maintained.

Methods

Specifications:

- 1 x 2D control: T25 flask.
- 1 x STR_{Ref}: Conventional stirred-tank bioreactor with one pitched blade impeller and open-pipe sparger.
- 1 x Comfy*Cell*$_{mini}$ (image left).
- Microcarrier: 10 g/L Synthemax II (Corning, USA).
- Cultivation method: Perfusion > 0.7 vessel volumes per day.
- VCD seeding = 2.5E+05 cells/mL, Vb > 97.0 % (direct inoculation).
- Process time: 7 days.

Results

Human induced pluripotent stem cells (iPSCs) were successfully expanded using the Comfy*Cell*$_{mini}$ system, achieving an expansion factor of 65. In benchmark comparisons with a conventional STR used as a negative (sparged) and as a positive control (headspace only), the Comfy*Cell* demonstrated superior performance, yielding over 1 B viable cells in total after only 7 days (see Fig. 5). In addition to the high expansion factor, the cultured cells retained their pluripotency markers and exhibited viability suitable for downstream applications and differentiation. Furthermore, successful differentiation into all three germ layers (ectoderm, mesoderm, and endoderm) could be demonstrated (see Fig. 6).

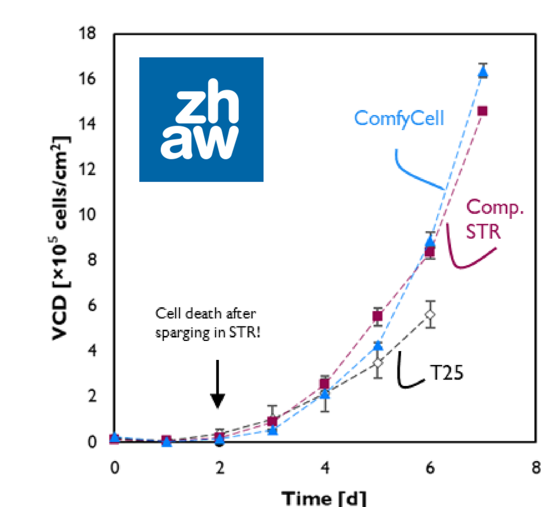

Figure 5 Viable cell density on the surface of the microcarriers in cells cm^{-2} during a 7-day iPSC expansion process.

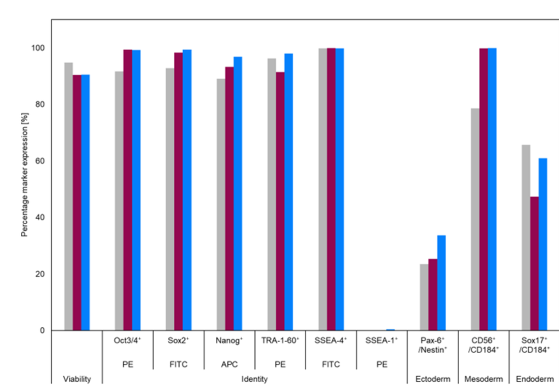

Figure 6 Identity and differentiation profile of the expanded iPSCs harvested from a T25 (control, grey), conventional STR (purple), and the Comfy*Cell*$_{mini}$ (blue).

Deep-dive: iPSC-derived progenitor NK cell expansion

Dr. Daniel Terheyden-Keighley, Yasemin van Heuvel, Moritz Meyer, Patrick Bongartz, Boris Greber
Catalent Inc., Berghausener Str. 98, 40764 Langenfeld (Rheinland), Germany

Introduction

Allogenic immune cell therapies present a promising approach to treating cancer. Here, allogenic natural killer cells (NKs), derived from healthy donors, umbilical cord or iPSCs, serve as especially potent candidates due to their cytotoxicity, broad tumor recognition, and immunomodulatory capacities. This provides a significant advantage when suppressing Graft-Versus-Host Disease (GVDH), when compared to T cells, positioning allogenic NK cells as ideal candidates for large-scale, off the shelf production under dynamic conditions [4].

This application note presents a first of its kind POC study providing preliminary insights into the linear scalability of human iPSC-derived progenitor natural killer cell (iNK) cultivation processes within our bionic Comfy*Cell* at 250 mL and 2 L scale.

1. Direct scale-up from 100 mL to 2 L in a first POC run!
2. A total fold expansion of > 38 was reached within the Comfy*Cell*$_{mini}$
3. More than 4.6 B total viable cells were reached within the 2 L Comfy*Cell*!
4. No oxygen limitations due to a similar $k_L a$ at both scales.

Methods

Specifications:

- Working vol.: 0.25 and 2 L
- 1 x Comfy*Cell*$_{mini}$ and 1 x Comfy*Cell* 2 L (image left)
- Cultivation method: Fed-Batch
- VCD seeding = 2.80E+4 cells mL^{-1}
- Process time: 11-20 days

Results

IPSC-derived progenitor NK cells were successfully expanded in our 250 mL Comfy*Cell*$_{mini}$ bioreactor, demonstrating robust proliferation over a 11-day cultivation period. The initial seeding density of 28'000 cells mL^{-1} increased to 1.3 M cells mL^{-1} by the end of the expansion phase. This corresponds to a total cell count exceeding 250 M, achieving a fold expansion of more than 38.

Direct scale-up from 100 mL to 2 L was easily achieved, reaching a total fold expansion of > 300, with 4.6 B total viable cells harvested following a 19-day cultivation process. These results highlight the efficiency of the Comf*Cell* bioreactor system in supporting NK cell proliferation, making it a suitable platform for large-scale cell therapy manufacturing.

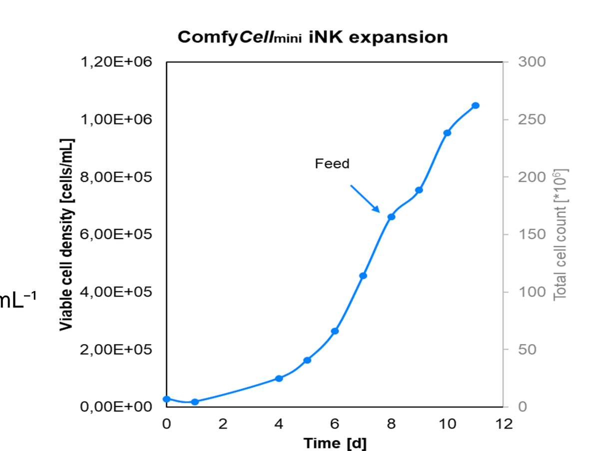

Figure 7 Cell growth kinetics of NKs within the Comfy*Cell*$_{mini}$ over a 11-day cultivation period.

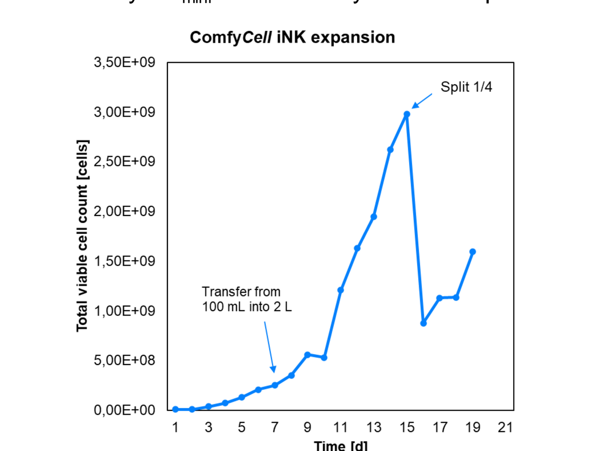

Figure 8 Cell growth kinetics of NKs following transfer from a 100 mL preculture to a 2 L Comfy*Cell* over a 21-day cultivation period.

References:
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[4] Berrien-Elliott, M.M., Jacobs, M.T. and Fehniger, T.A. (2022) 'Allogeneic natural killer cell therapy', Blood, 141(8), p. 856. Available at: https://doi.org/10.1182/BLOOD.20 22016200.