BIOTHRUST

In cooperation with

Universitätsmedizin Essen
Universitätsklinikum

IFZ INSTITUT FUR ZELLBIOLOGIE (Tumorforschung)

Proof-of-Concept Study

Evaluation of BioThrust's Mem*Stir*: Human Lung Organoid expansion in 2 L

Introduction

Respiratory diseases are among the leading cause of mortality globally and thus associated with a substantial medical burden and costs highlighting the need for additional translational research. *In vitro* models, from basic airway cells to advanced tissue-engineered human lung organoids (HLOs), are vital for studying respiratory conditions such as infections, chronic inflammation, and cancer. Lung organoids, derived from somatic stem cells or human-induced pluripotent stem cells (hiPSCs) in particular, provide crucial insights into lung injury and regeneration by realistically mimicking *in vivo* human tissues [1-2].

This application note presents a Proof-of-Concept (POC) study showcasing preliminary results in the development of scalable lung organoid cultivation processes. A key innovation is the novel bionic bioreactor, the Comfy*Cell*, and the novel membrane stirrer (Mem*Stir*) developed by BioThrust. The artificial lung was designed for use in Stirred-Tank Bioreactors (STRs) and offers low shear rates and exceptional oxygen supply through diffusion, eliminating gas bubbles and thus foam formation [3]. These conditions enable the scalable and more physiological production of shear-stress sensitive organoids in a 3D setup.

Objective

In a collaboration with Prof. Dr. Diana Klein from the University Hospital Essen (UKE), hiPSC were expanded in 2D and differentiated into lung organoids by embryoid bodies (EB) generation and subsequent branching lung organoid (BLO) media treatment, following previously published protocols [4, 5]. The goal of this project was to investigate whether using the Mem*Stir-driven* bioreactor is feasible for 'automated' differentiating HLOs in a 3D format and at a 2 L scale (POC experiments). Therefore, pre-differentiated EB were transferred to the Comfy*Cell* bioreactor and cultured under a contolled condition for consecutive 28 days (Fig. 2).

Key Results

1. Successful scale-up from 30 mL to 2 L: POC + validation.
2. Improved oxygen supply & homogenous flow dynamics.
3. Comparable lung organoid maturity to control & robust cultivation for up to 35 days.
4. Maintained homogeneity without compromising the integrity of lung organoids.

Materials & Methods

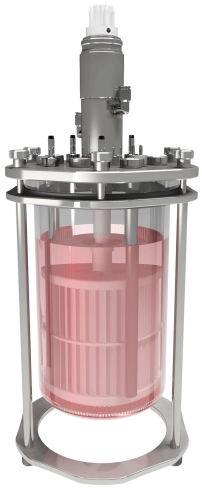

Figure 1: Front view of the 2 L Mem*Stir* bioreactor vessel.

Specifications

- Bioreactor:
  Total vol.: 2.5 L
  Working vol.: 2 L
- 1 x Mem*Stir* 2 L (Image top)
- Cultivation method: Batch
- Seeding density:
  VCC = 6.25E+3 cells/mL
  Vb: > 90%
- Process time: 28-35 days

Parameter	Mem*Stir*
Stirrer	40 rpm
DO setpoint	50 %
Sparger gassing	0.5 L/min constant flow, Air + 5 % CO2 on demand, Open-loop off-gas circulation
Oxygen transfer rate (OTR)	0.6 (0.8) mmol/L h
pH-Range	7.4 – 7.55
Osmolality-Range	310 – 350 mOsm/kg

Abbreviations

POC = Proof of concept
Mem*Stir* = Membrane stirrer
STR = Stirred-tank bioreactor
hiPSC = human induced pluripotent stem cells
AFE = Anterior Foregut Endoderm
pHLO = premature human lung organoids
EB = Embryoid body
VCC = Viable cell concentration
Vb = Cell viability
rpm = rounds per minute
DO = dissolved oxygen
OTR = O_2 transfer rate

Results

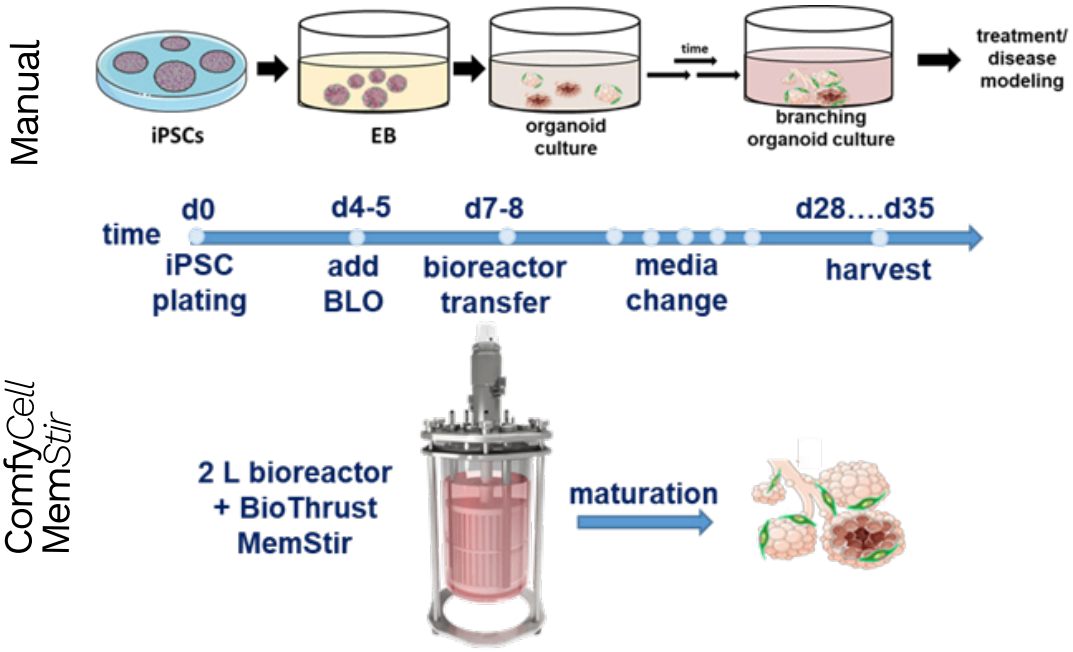

Figure 2: Process overview for the differentiation and maturation of hiPSC-derived lung organoids in a 3D system using BioThrust's MemStir. Created with BioRender.com.

During HLOs cultivation and differentiation within 35 days, expansion and differentiation was easily achieved using a specialized 2D to 3D expansion and differentiation process using the ComfyCell bioreactor (Fig. 2). HLO growth was monitored by simple photographs, indicating an continuous increase in organoid volume (Fig. 3).

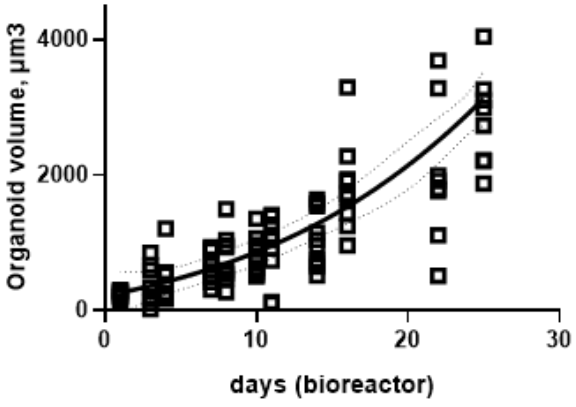

Figure 3: Organoid diameter during Mem*Stir* cultivation. Control images were taken weekly during cultivation. Exemplary images from samples on day 14 are shown on the right.

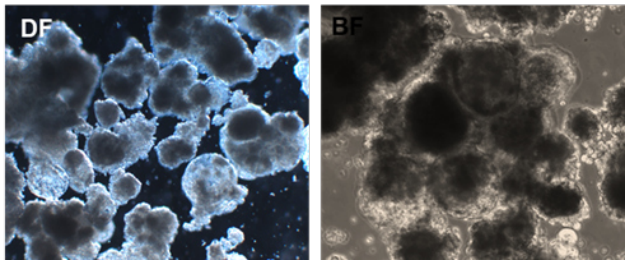

Figure 4: Microscopic images taken from samples on day 35 (experiment end) are shown. BF bright filed; DF dark field..

Morphological analyses by stereo microscopy (Fig. 4), phase contrast microscopy and immunohistochemistry (not shown) further confirmed the efficient generation of airway and alveolar structures representing the desired destination tissue. Post-process single-cell transcriptomics revealed efficient differentiation including epithelial and mesodermal lung cells as highlighted by the distribution of epithelial EpCAM/ CD326, ECAD/CDH1, mesodermal PDGFRB and COL1A1 expressions as well as proximal-distal (SOX2-SOX9) patterning (Fig. 5). Clustering of the single cells revealed the presence of 15 different main cellular subsets in both control and Mem*Stir*-driven Comfy*Cell* processes (Fig. 6). Of course, the total cellular outcome was also higher within the Mem*Stir*.

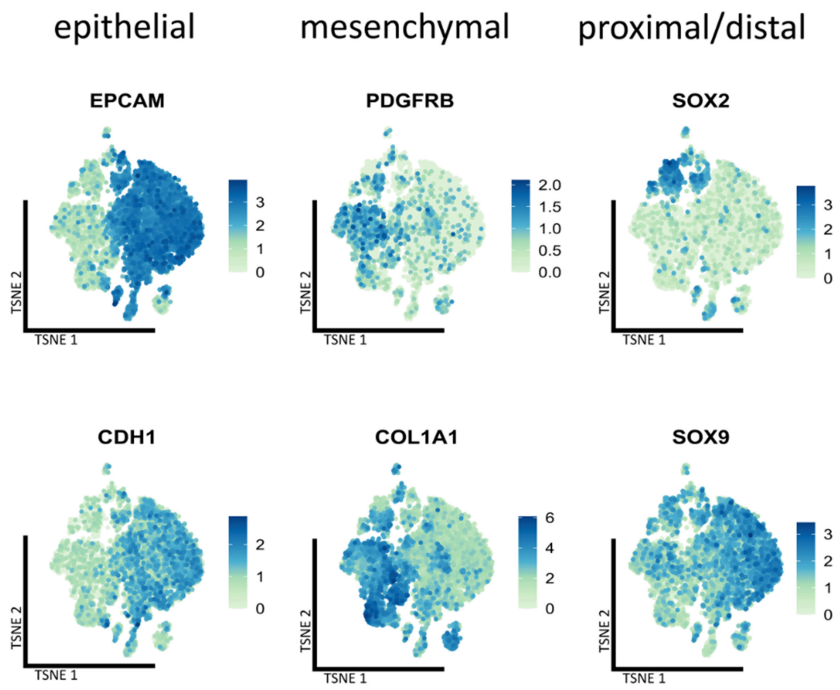

Figure 5: Images from computational analysis of living single-cell transcriptomes within the organoids of the MemStir-driven bioreactor, Comfy*Cell*, cultures versus manually generated ones (control). Expression map of representative epithelial (EPCAM, CDH1), mesenchymal (PDGFRB, COL1A1) marker genes, or SOX2 and SOX9 for proximal-distal patterning in all clusters.

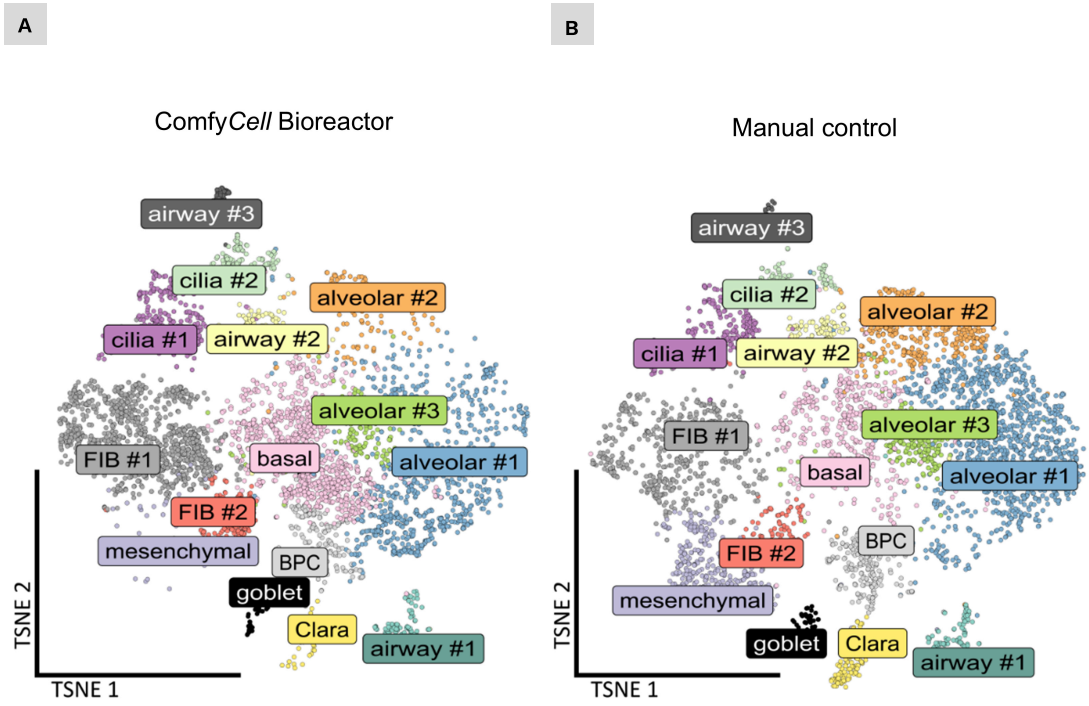

Figure 6: Images from computational analysis of living single-cell transcriptomes within the organoids of the MemStir-driven bioreactor, the Comfy*Cell*, versus manually generated ones (control). UMAP of all single cells colored by identified clusters in LuOrgs either bioreactor-generated (A) or manually (B). One dot represents one single cell transcriptome.

Conclusion

- Highly promising lung organoid differentiation and maturation in a 3D setup.
- Successful validation of POC.
- Single-cell transcriptomics identified similar lung cell clusters as present in human clinical lung samples.
- Further experiments are ongoing. (Direct iPSC cultivation and differentiation.)
- Future production in 3D and at industrial scale feasible.

Discussion

Mammalian cell cultivations in conventional stirred-tank bioreactors face various limitations such as foam and shear stress from bubble-aeration and mechanical agitation, insufficient oxygen transfer, nutrient gradients, and scalability challenges, negatively impacting cell viability and productivity. BioThrust's bionic bioreactor addresses these issues by minimizing shear forces, eliminating foam, reducing culture time, and enhancing medium efficiency. The Comfy*Cell* is suitable for various cell types and process modalities, from suspension to adherent cells, and from expansion to *in situ* differentiation.

Within 35 days, the Comfy*Cell* bioreactor enabled efficient 2D-to-3D expansion and differentiation of human lung organoids (HLOs), producing airway and alveolar structures confirmed by microscopy and immunohistochemistry. Single-cell transcriptomics revealed robust epithelial, mesodermal, and proximal–distal lung patterning, with 15 distinct cell subsets resembling those in human clinical lung samples. The Mem*Stir*-driven process yielded higher total cell output, validating proof-of-concept and highlighting the potential for scalable 3D lung organoid production from iPSCs.

Real process scalability is achieved by geometric analogy, enabling linear scale-up from 250 mL to 200 L. The Mem*Stir* ensures low shear forces, uniform mixing, and precise process control for consistent product quality especially suitable for mammalian cell cultivations in 3D as shown in this application note with human iPSC-derived lung organoids.

References

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