BIOTHRUST

In cooperation with

UNIVERSITY OF COLOGNE

Proof-of-Concept Study

Evaluation of BioThrust's Mem*Stir*: iPSC-derived Cardiomyocyte expansion in 2 L

Introduction

BioThrust is creating innovative, bionic membrane stirrer (Mem*Stir*) for the direct expansion of (stem-) cells and cell-derived spheroids, aggregates as well as organoids in bioreactors [1]. This application note constitutes a Proof-of-Concept (POC) preliminary study aiming at the development of a scalable production process for organoid-based heart cell therapies.

In this initial POC, Prof. Kurt Pfannkuche's laboratory at the University of Cologne leveraged its expertise in the cultivation and differentiation of induced pluripotent stem cell (iPSC-)-derived cardiomyocytes (iPSC-CM) into functional and transplantable aggregates. These CM-aggregates can serve as "microtissues" and potentially replace cardiac tissue loss after myocardial infarction (MI). The treatment of a MI requires approx. 8 billion cells per dose, which means an annual global cell requirement of more than 24 quadrillion cells. This represents a significant bottleneck when using conventional 2D production systems [2 - 3]. In this preliminary study a scalable process using BioThrust's Mem*Stir* in a 2 L bioreactor setup was investigated. The project's goal was to establish a POC for expanding & differentiating iPSC-derived embryoid bodies to functional cardiomyocytes in a 3D and 2-liter scale.

Objective

- POC for 3D differentiation and expansion of iPSC-CMs over 7 days using BioThrust's Mem*Stir* at a 2 L scale.
- Evaluation of the membrane-gassing as well as fluid dynamics on iPSC-CM differentiation.
- Determination of CM functionality after 3D Mem*Stir* cultivation.

Key Results

1. Direct scale-up from 14 mL to 2 L (3D) in a first POC reaching > 200 million functional CMs.
2. *In situ* differentiation from EBs to "beating" CMs within 7 days & a viability > 90 %.
3. Enhanced oxygen supply and homogeneous flow dynamics.
4. No Antifoam needed.

Materials & Methods

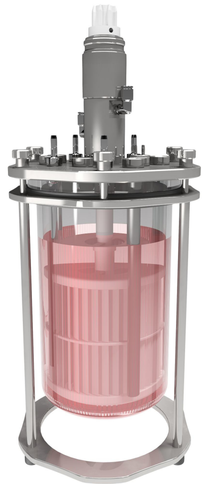

Figure 1: Front view of the 2 L Mem*Stir* bioreactor vessel.

Specifications

- Bioreactor size: 3 L;
  Working volume: 2 L.
- Consumable: 1 x Mem*Stir* 2 L (See Fig. 1).
- Cultivation method: Batch.
- Seeding of embryoid bodies (EBs): 3.00E+05 EBs, 98 % Viability (Vb).
- Process time in 3D: 7 days

Parameter	Mem*Stir*
Stirrer	62 rpm
DO	>20 %
pH	7.0 ± 0.2 (CO_2 adjusted)
Headspace gassing	-
Membrane gassing	1.95 mL/min constant air flow; 0.05 mL/min CO_2
Antifoam	-

Abbreviations

Mem*Stir* = Membrane stirrer
STR = Stirred tank bioreactor
Vvm = volume of air sparged per working volume per minute
CM = Cardiomyocyte
DO = Dissolved Oxygen
VCC = Viable cell concentration
Vb = Cell viability
EB = Embryoid Body
iPSC = induced Pluripotent Stem Cell

Preliminary Results

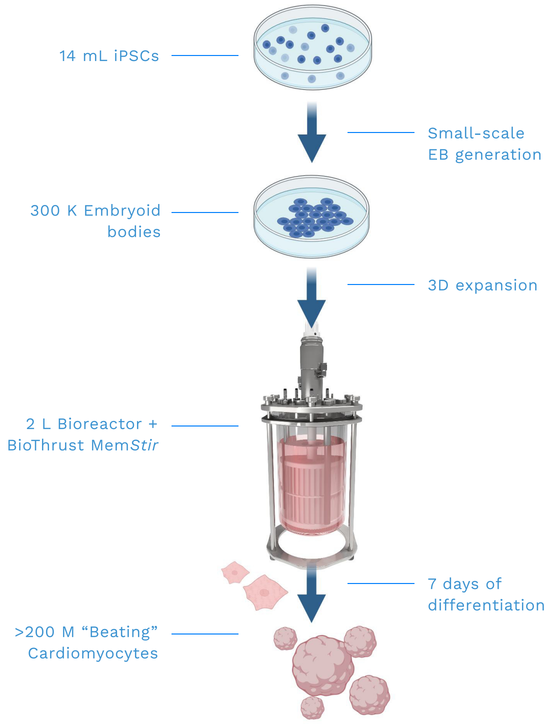

Figure 2: Process overview for the generation of iPSC-derived cardiomyocytes from small-scale to 2 L 3D using BioThrusts Mem*Stir*. Created with BioRender.com.

Induced pluripotent stem cells (iPSCs) are initially cultured in a two-dimensional (2D) or small-scale 3D environment to form spherical cell aggregates known as embryoid bodies (EBs) within two days. These EBs are then transferred to a bioreactor, where differentiation into cardiomyocyte-aggregates occurs during a 7-14 days cultivation period (Fig. 2). The differentiation of murine iPSCs happens spontaneously whereas differentiation of human iPSCs (hiPSCs) must be done by induction. To induce mesodermal differentiation of human iPSCs, a specific molecule is administered, the Wnt glycoprotein. This step is followed by a specific molecule (CHIR 99021) administered to promote cardiac differentiation. Subsequently, the differentiating cells are exposed to a series of growth factor cocktails to promote the formation of cardiovascular cell types, e.g., cardiomyocytes and endothelial cells, resulting in functional CM-aggregates. This technology was optimized by the working group of Prof. Dr. Pfannkuche making it applicable for large-scale *in situ* differentiation and expansion experiments. A future 2 L Mem*Stir* expansion using these hiPSCs is planned for the future.

During an initial 2 L batch run using the BioThrust Mem*Stir*, murine EB expansion and differentiation to functional CM-aggregates was achieved within only 7 days. Over 200 million functional, beating cardiomyocytes were successfully generated during the initial two-liter iPSC-CM differentiation process.

Figure 3 illustrate microscopic images of the CM-aggregates morphology at harvest day respectively. In contrast, a parallel experiment in a conventional stirred-tank bioreactor did not produce viable CMs (data not shown). A representative photograph of the 2 L Mem*Stir* bioreactor is shown in figure 4.

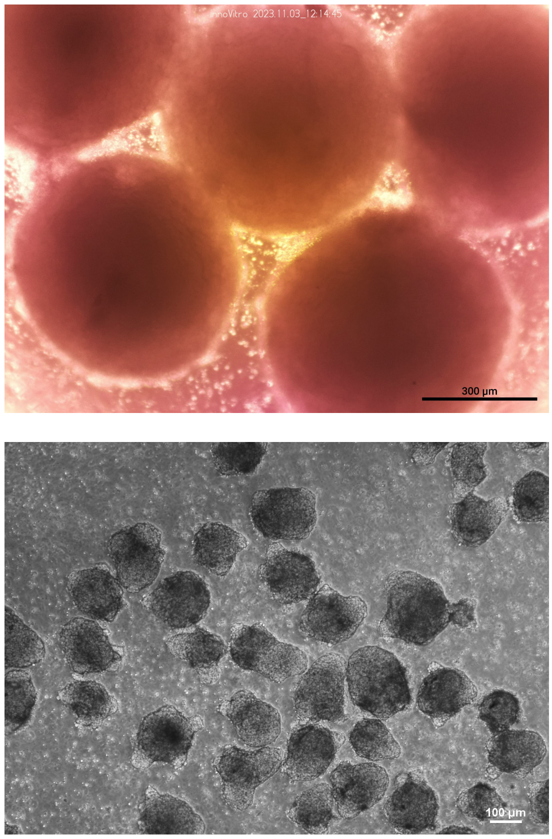

Figure 3: Microscopic images of murine iPSCs-CM aggregates visualized by InnoVitro GmbH (top) and the Pfannkuche lab at the University of Cologne (bottom).

Figure 4: iPSC-derived EB expansion and differentiation to cardiomyocyte-aggregates within the 3 L Mem*Stir*-driven bioreactor at the Pfannkuche Lab at the University of Cologne.

Discussion & Outlook

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 Mem*Stir* addresses these issues by minimizing shear forces, eliminating foam, reducing culture time, and enhancing medium efficiency. This system is suitable for various cell types and process modalities, from suspension to adherent cells, and from expansion to *in situ* differentiation. 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 iPSC-CMs.

In the future, this consortium will focus on investigating the maximum amount and density of human and animal iPSC-CMs that can be successfully cultivated in this system by direct 2D to 3D scale-up and differentiation. It is expected that gassing through the BioThrust Mem*Stir* bioreactor will lead to a substantially higher loading density as well as faster cell proliferation compared to 2D, shake flasks or conventional STRs using bubble-aeration. Future *in vivo* preclinical pilot trials in a pig heart attack model are planned to validate transplant ability and functionality. These preliminary as well as future results will foster the applicability in 3D clinical iPSC-CM manufacturing at scale.

References

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