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Microcarrier-based Expansion of Human Mesenchymal Stem Cells in the BIOSTAT STR® 50L

Application Note

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turning science into solutions

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Purpose

This note describes the culture conditions and setpoints for expansion of human bone marrow-derived mesenchymal stem cells (hBM-MSCs) on microcarriers in the Sartorius BIOSTAT STR® 50L, single-use stirred tank reactor. MSCs can be produced in bulk in clinically relevant numbers, viability and phenotype in the BIOSTAT STR®.

Introduction

Human bone marrow-derived mesenchymal stem cells (hBM-MSCs) have potential to be developed as cell therapeutics for the treatment of Parkinson's disease, type I diabetes, arthritis, burn victims and cardiovascular diseases[1-7].

Until now, the majority of MSCs for clinical trials have been expanded in planar, static cultivation systems (e.g. stacked plate systems). However, these systems have limitations in terms of generating clinically relevant numbers of MSCs, which typically range between 0.5 and 5 × 10^6 cells/kg. While single-use stirred bioreactors were originally developed for the cultivation of conventional mammalian cell lines (e.g. CHO), MSC expansion on microcarriers in these systems has now been demonstrated, providing an alternative to traditional planar and static cultivation systems[8].

This application note highlights that the BIOSTAT STR® 50L is a suitable system for the serum-reduced expansion of clinically applicable numbers of hBM-MSCs.

Materials and Methods

Cells, medium and microcarriers

Cryopreserved bone marrow-derived MSCs (Lonza Cologne GmbH, Germany) from a single consenting and informed donor (second passage, PDL=10) were used for the cultivation in the BIOSTAT STR® 50L. For inoculation, an average cell number of up to 109 MSCs from a 4-day culture (2-layer stacks) was used. The cells were cultivated in a specially developed, serum-reduced (5 % FBS) medium (Lonza) in combination with Cultispher®-G microcarriers (GE) at concentrations of 0.3 % solid fraction. Microcarrier preparation was carried out according to the manufacturer's recommendations[9-12].

Cultivation setup

The hBM-MSC cultivation process in the BIOSTAT STR® 50L was divided into 3-steps: (1) initial cell attachment, (2) cell expansion and (3) cell harvest (Figure 1).

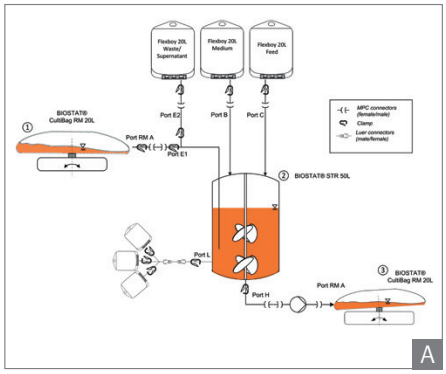

Figure 1: Experimental setup of the cultivation process in the BIOSTAT STR® 50L. A) Schematic overview of the 3-step cultivation process with cell attachment phase (1: BIOSTAT® CultiBag RM 20L), cell expansion (2: BIOSTAT STR® 50L) and cell harvest (3: BIOSTAT® CultiBag RM 20L). The BIOSTAT STR® 50L was equipped with two segment blade impellers (SBI) and a ring sparger. B) Experimental setup at the Zurich University of Applied Sciences (ZHAW).

In brief, the following protocol was used for hBM-MSC culturing in the BIOSTAT STR® 50L:

Day -4: Inoculation of 2-layer stacks with cryopreserved MSCs (pre-culture: 37 °C, 80 % humidity, 5 % CO_2).

Day -1: Preparation and sterilization of the macroporous degradable microcarriers (0.3% solid fraction). Installation of the BIOSTAT STR® 50L bag equipped with two segment blade impellers (SBI) and a ring sparger in the stainless steel holder followed by filling of the bag with 35L of medium. Equilibration of the optical sensor patches for pH and DO measurement.

Day 0: Equilibration of the microcarrier | medium in a BIOSTAT® CultiBag RM 20L bag. Inoculation of the bag with 1.75×10^8 MSCs from 2-layer stack followed by a 16 hour cell attachment period before transferring the MSC culture to the BIOSTAT STR® 50L.

Day 5: Feeding with specially developed feed solution (15 L)

Day 1-7: Sampling, analytics and recalibration of the online pH and DO sensors.

Day 7: Transfer of the culture suspension into a BIOSTAT® CultiBag RM 20L for cell harvest.

Sampling and quality control

During the course of the cultivation, 40 mL samples were taken daily using single-use manifold bags (Sartorius Stedim Biotech). Metabolites were measured with a CedexBio (Roche Diagnostic) and a BioProfile (Nova Biomedical). Cell densities were measured with a NucleoCounter® NC-100™ (Chemometec). In addition, flow cytometric surface marker analysis with fluorochrome-conjugated anti-human CD34, CD45, CD73, CD90 and CD105 (eBiosciences) was performed at the time of MSC inoculation and post-harvesting to assess MSC viability and phenotype.

Results and discussion

We evaluated the time-dependent MSC expansion profile in the BIOSTAT STR® 50L (Figure 2A). As a control, MSCs were also cultured in two spinner flasks. Through previous investigations, the optimum cell harvest point was defined to be on day 7, at which point the macroporous microcarriers were completely covered with MSCs but had not formed aggregates (Figure 2B). At the time of harvest, a maximum living MSC density of $7.16 \pm 0.68 \times 10^5$ cells/mL was achieved in the BIOSTAT STR® 50L corresponding to a 51 fold expansion. In addition, the MSCs grew with a mean specific growth rate (μ) of 0.68 d^{-1}. No nutrient or oxygen limitations were observed throughout the cultivation process. While comparable mean growth rates of 0.71 d^{-1} and 0.67 d^{-1} could be reached in the control spinner flasks A and B respectively, slightly lower maximum living cell densities were achieved in these vessels ($5.3 \pm 0.2 \times 10^5$/mL and $6.6 \pm 0.5 \times 10^5$/mL).

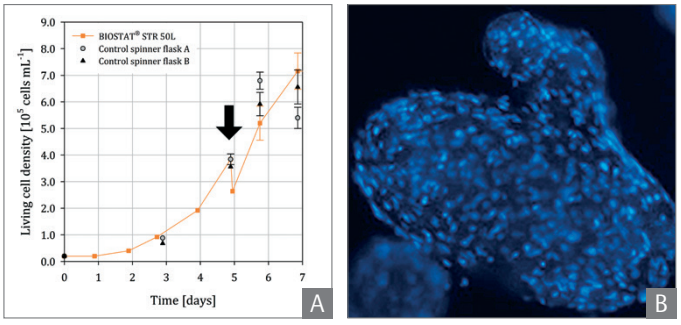

Figure 2: Profiles of cell densities (A) and DAPI-staining during hBM-MSC cultivation. (A) The arrow indicates feeding after 5 days of cultivation. (B) Completely covered macroporus microcarrier with hBM-MSCs.

Flow cytometry analysis using cell samples obtained from the seed inoculum as well as post-harvesting revealed that the mesenchymal stem cell markers $CD73^+$, $CD90^+$, $CD105^+$ were highly expressed (≥ 98%) at both time points while the negative, hematopoietic markers $CD34^-$, $CD45^-$ were not detectable (Figure 3). In summary, these results demonstrate that the BIOSTAT STR® 50L enables scalable bulk production of $3.58 \pm 0.69 \times 10^{10}$ MSCs in a closed single-use system with quality parameters equivalent to those achievable by commonly used small scale systems such as T-flasks.

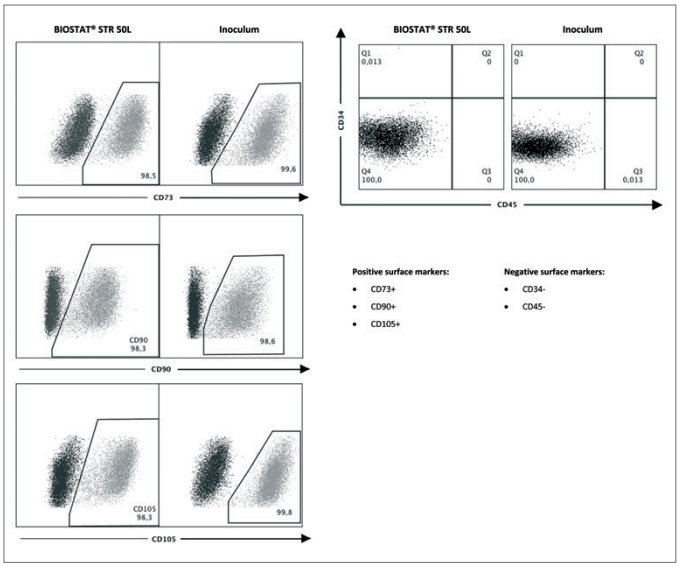

Figure 3: Flow cytometric analysis of hMSC surface marker expression. Samples were analyzed before (inoculum) and at the end (harvest) of the expansion process in the BIOSTAT STR® 50L. Positive surface markers: $CD73^+$, $CD90^+$, $CD105^+$. Negative surface markers: $CD34^-$, $CD45^-$.

Conclusion

The overall results underscore that the BIOSTAT STR® 50L is well suited for the expansion of MSCs. Clinically relevant numbers can be generated without undesired cell differentiation as demonstrated by surface marker expression profiling. Additional increases in cell yield could be achieved by further optimizing microcarrier density and | or the feeding strategy.

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