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Microcarrier-Based Expansion of Human Mesenchymal Stem Cells in the Univessel® SU

Anita Ott[1], Valentin Jossen[1], Ann Siehoff[2], Christian van den Bos[2], Dieter Eibl[1], Regine Eibl[1]

[1] Zurich University of Applied Sciences, School of Life Sciences and Facility Management, Institute of Biotechnology, Wädenswil, Switzerland

[2] Lonza Cologne GmbH, Cologne, Germany

Correspondence
Email: bioprocess.support@sartorius.com

Abstract

This note describes the culture conditions and setpoints for expansion of human adipose-derived mesenchymal stem cells (hAD-MSC) on microcarriers in the Sartorius Biostat® 2 L single-use Univessel. Typically, such culture conditions can be scaled to 50 L and larger Biostat STR® reactors. Reliable, scalable expansion of MSCs is critical for their use as a therapeutic agent.

Introduction

The increasing number of clinical trials with human mesenchymal stem cells (MSCs) and progress in regenerative medicine[1,2] has created a need for higher quantities of these cells at the desired quality. For autologous therapies, a minimum of 5×10^8 cells are required for one single dose[2]. It has already been demonstrated that MSCs can successfully be grown on microcarriers in single-use stirred bioreactors at benchtop-scale instead of the commonly used planar, one- or multiple-layer flasks, such as CellSTACKs or Cell Factories[3–6].

Medium, Methods and Equipment

Overview of Set-up Procedure

Day –1
Preparation of SoloHill Pronectin F (0.75% solid fraction) as well according to the manufacturer's recommendation.

Day 0
Installation of the Univessel® SU bioreactor. Transfer of the microcarriers into the bioreactor vessel and filling with 0.7 of custom-made MSC medium. Equilibration of the optical sensor patches for pH and DO measurement for 4 hours.

Thawing and inoculation with a seeding density of 1.5×10^7 MSCs (pooled cells from cryo-preserved vials). 4-hour cell attachment phase without agitation. Filling up to 2 L working volume with medium. Starting agitation at 100 rpm.

Day 0 – 7
Sampling, analytics and recalibration of online pH and DO sensors. Instead of the sampling device depicted in Fig. 1, single-use manifold bags were used (see also[5]).

Day 4
Exchange of 50% of medium with fresh medium.

Day 7
Cell harvest by separation of the microcarrier-cell aggregates from the medium, enzymatic cell detachment, cell resuspension, vialing and freezing.

Cells, Medium and Microcarrier

Cryopreserved adipose-derived MSCs (Lonza Cologne GmbH, Germany) from a single consenting and informed donor (second passage, PDL = 10) were used for inoculation, with a recommended seeding number of 1.5×10^7 cells. The cells were cultivated in a serum-reduced (5% FBS) medium. The required amount of SoloHill ProNectin F microcarriers (0.75% solid fraction) was prepared according to the manufacturer's specifications.

Cultivation Setup

The cultivation process of the MSCs in the Univessel® SU includes 3 steps: (1) initial cell attachment, (2) cell expansion, and (3) cell harvest. The microcarriers along with 0.7 L of medium were transferred to the Univessel® SU and equilibrated for 4 hours at 37 °C, 5% CO_2, 85 rpm. Afterwards, the thawed and pooled MSCs were inoculated at an initial density of 2.8×10^3 cells cm^{-2}. No agitation was performed for 4 h to allow the cells to attach to the microcarriers. The Univessel® SU was then filled with medium to the maximal working volume of 2 L. The impeller speed was initially set to 100 rpm and increased to 135 rpm as cultivation progressed. The MSCs were grown at 37 °C, pH 7.2 and 0.1 vvm headspace aeration for 7 days. On day 4 of cultivation (cell density between 2 and 3×10^4 cells cm^{-2}), a 50% medium exchange was performed to prevent nutrient limitation. Once the maximum cell density was reached (day 7 of cultivation), the cells were separated from the microcarriers by using a sieving procedure combined with enzymatic cell detachment and washing steps[5]. Finally, the microcarrier-free suspension was centrifuged at 2000 *g* for 8 min, followed by supernatant removal and cell resuspension in fresh culture medium before vialing and freezing occurred.

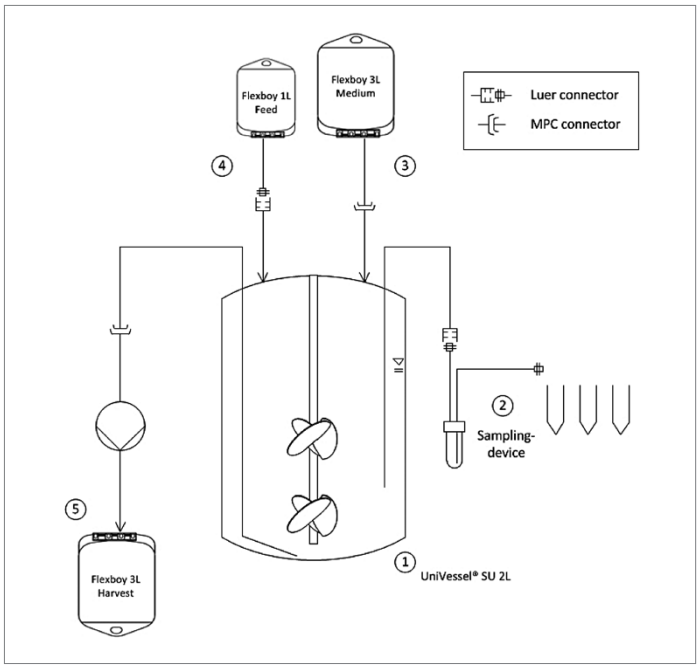

Figure 1: *Experimental Setup of the Cultivation Process in the Univessel® SU 2 L.*
Schematic overview of the experimental setup. The Univessel® SU 2 L (1) is additionally equipped with a sampling device (2), medium bag for medium feed after cell attachment (3), feeding bag for partial media exchange on day 4 (4), and harvest bag for cell harvest on day 7 (5).

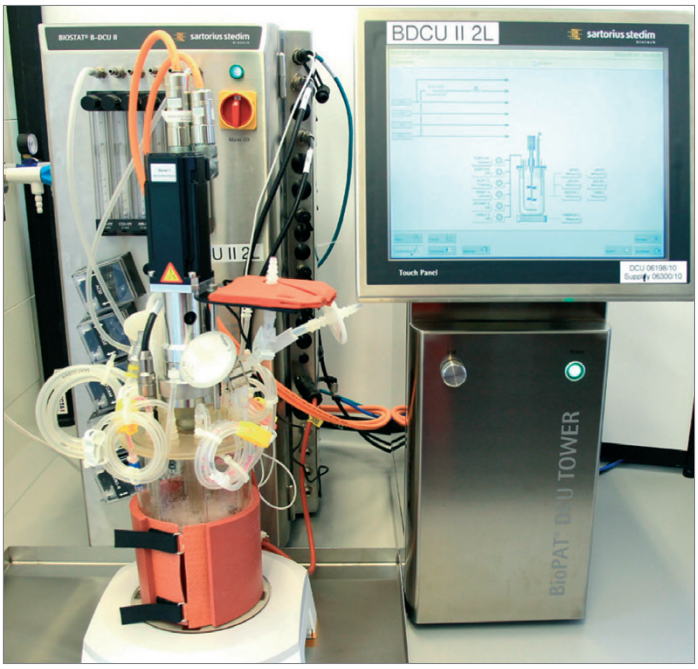

Figure 2: *Experimental Setup at the Zurich University of Applied Sciences (ZHAW).*

Culture Conditions

Working volume	2 L
Microcarrier concentration	0.75% microcarrier solid fraction
Inoculated cell number	1.5×10^7 MSCs
Agitation speed	100 rpm (below NS1u) and 135 rpm (NS1)
Temperature	37 °C
Head aeration	0.1 vvm
Dissolved oxygen (DO)	> 20%
Cultivation time	7 days

Sampling and Quality Control

A daily sample of approximately 25 mL was taken with a sampling device to measure metabolites with the CedexBio (Roche Diagnostic) and | or the BioProfile (Nova Biomedical) as well as cell densities with the NucleoCounter® NC-100™ (Chemometec). Furthermore, a staining of the microcarrier-cell suspension with 4,6-diamidino-2-phenylindole (DAPI) was performed to evaluate carrier colonization and aggregate formation. In order to check the quality of the MSCs after cell harvest, flow cytometric investigations with fluorochrome-conjugated anti-human CD34, CD45, CD73, CD90 and CD105 (eBiosciences) were carried out. The results were compared to the surface marker profiles of the inoculated cells.

Results

Figure 4 exemplarily shows a typical time-dependent profile of the cell growth in the Univessel® SU bioreactor and control spinner flask. The comparability of the growth patterns in the two cultivation systems is evident. Within 7 days of expansion, a maximum cell number of $(5.3 \pm 0.5) \times 10^8$ MSCs was achieved in the Univessel® SU. This corresponds to an expansion factor of 35.4 ±0.4 and a population doubling time of (25.6 ±0.2) hr. On day 3, the cells were in a lag phase followed by an exponential growth phase until day 7. Glucose and lactate concentrations (data not shown) in the medium correlated well with cell growth. On day 4, a 50% media exchange was performed. DAPI staining of microcarrier-cell aggregates was performed on day 7, immediately before cell harvest. Notably, the microcarriers were completely covered with cells and formed aggregates of 2 mm in diameter. TrypLE Select-based detachment of the cells led to a harvest recovery of > 98% with cell viabilities exceeding 98%.

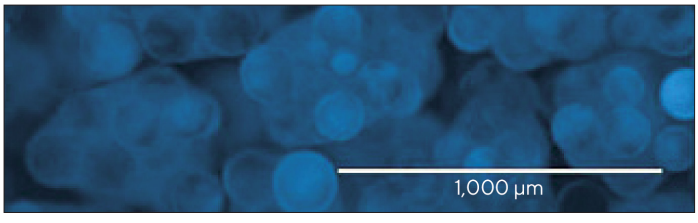

Figure 3: *Polystyrene-Based Microcarriers Completely Covered With MSCs (White Bar = 1000 µm).*

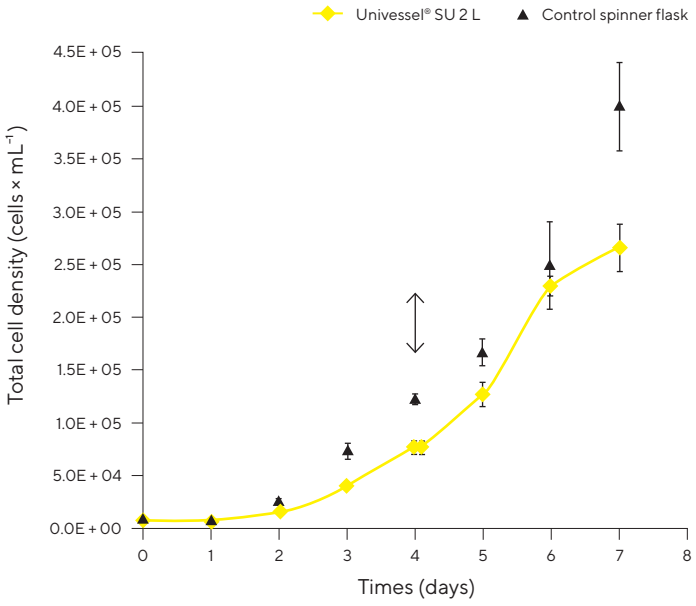

Figure 4: *Growth Courses of MSCs Expanded in the Univessel® SU Bioreactor and Control Spinner Flask and DAPI-Staining on Day 7 of the MSC-Cultivation.*
The arrow indicates the 50% medium exchange.

Cell Quality Control

Flow cytometric analysis of the MSCs (presence of $CD73^+$, $CD90^+$, $CD105^+$ and absence of $CD34^-$, $CD45^-$ surface markers) demonstrated that the phenotypic properties were maintained (data not shown).

Conclusion

The results demonstrate that the Univessel® SU bioreactor is suitable for the expansion of adipose-derived MSCs growing on microcarriers whilst maintaining the expected surface marker expression profile. Moreover, the Univessel® SU bioreactor can be used to generate clinically relevant doses for autologous cell therapy, typically in the order of 5×10^8 for a single dose.

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Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

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