sartorius stedim biotech

Microcarrier-based Expansion of Human Mesenchymal Stem Cells in the UniVessel® SU

Application Note

#05
#06
#07
#08
#09

turning science into solutions

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

Purpose

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® 2L 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 & 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 A, 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.

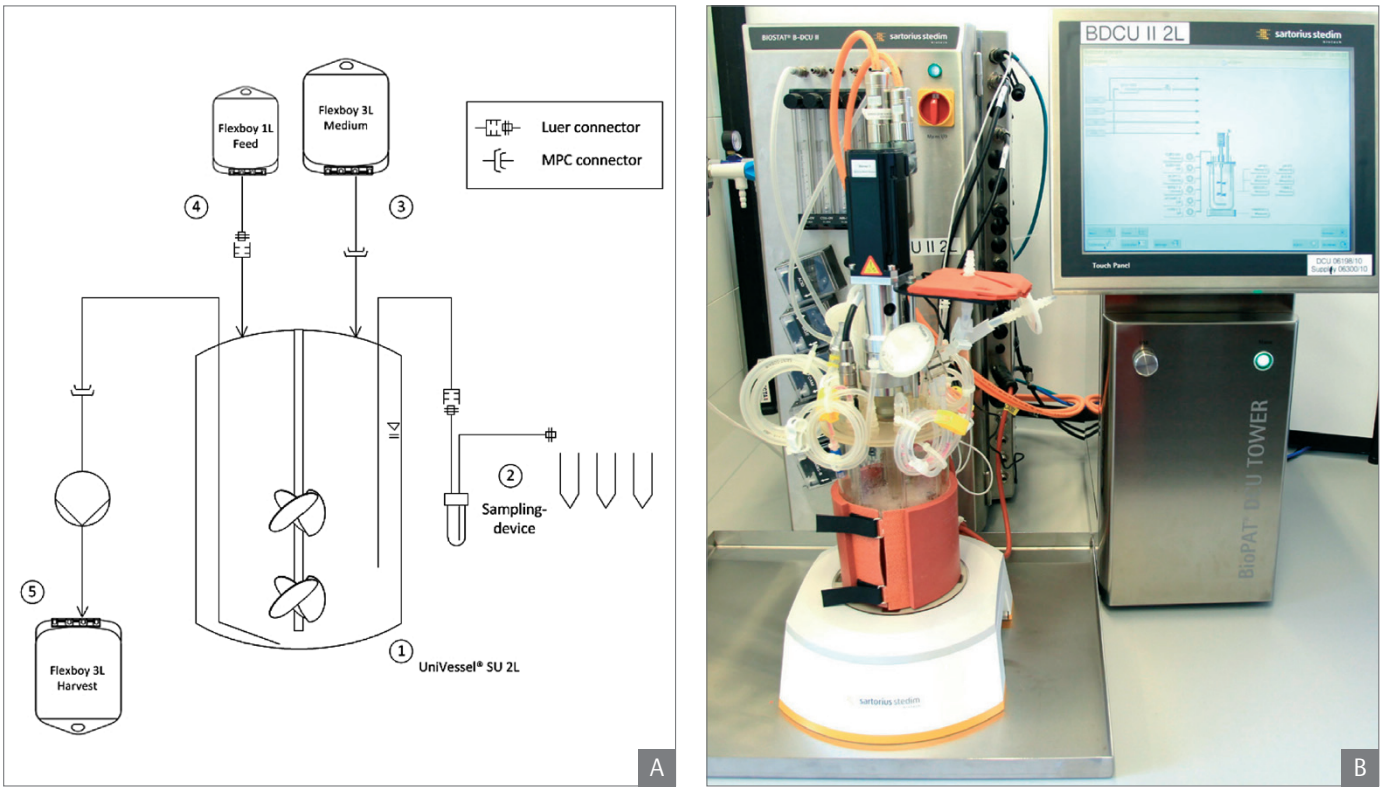

Figure 1: Experimental setup of the cultivation process in the UniVessel® SU 2L. A) Schematic overview of the experimental setup. The UniVessel® SU 2L (1) is additionally equipped with a sampling device (2), medium bag for medium feed after cell attachment (3), feeding bag for partial media exchange on day 4 (4), and harvest bag for cell harvest on day 7 (5). B) Experimental setup at the Zurich University of Applied Sciences (ZHAW).

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.

Culture conditions

Working volume:	2 L
Microcarrier concentration:	0.75 % microcarrier solid fraction
Inoculated cell number:	1.5×10^7 MSCs
Agitation speed:	between 100 rpm (below N_{S1u}) and 135 rpm (N_{S1})
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 2 A 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 ± 0.5) × 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) h. 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 %.

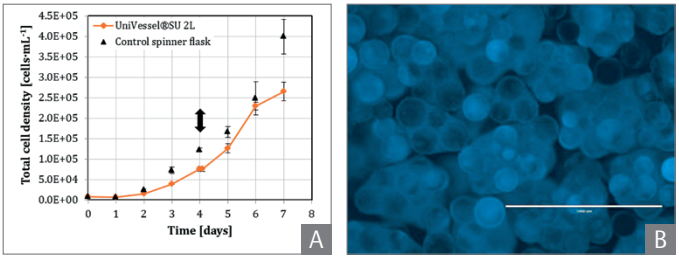

Figure 2: Growth courses of MSCs expanded in the UniVessel® SU bioreactor and control spinner flask (A) and DAPI-staining on day 7 of the MSC-cultivation. (A) The arrow indicates the 50 % medium exchange. (B) Polystyrene-based microcarriers completely covered with MSCs (white bar = 1000 µm).

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

References

1 Wang J., Liao L., Tan J. Mesenchymal-stem-cell-based experimental and clinical trials: Current status and open questions. *Expert Opin Biol Ther.* 2011;11(7):893-909.

2 Ren G., Chen X., Dong F., Li W., Ren X., Zhang Y., Shi Y. Mesenchymal stem cells and translational medicine: Emerging issues. *Stem Cells Transl Med.* 2012;1(1):51-58.

3 Cierpka K., Elseberg CL., Niss K., Kassem M., Salzig D., Czermak P. MSC production in disposable bioreactors with regards to GMP and PAT. *Chem Ing Tech.* 2013;85(1-2):67-75.

4 Jing D., Sunil N., Punreddy S., Aysola M., Kehoe D., Murrel J., Rook M., Niss K.. Growth kinetics of human mesenchymal stem cells in a 3-l single-use, stirred-tank bioreactor. *Biopharm Int.* 2013;26(4):28-38

5 Schirmaier C., Jossen V., Kaiser SC., Jüngerkes F., Brill S., Safavi-Nab A., van den Bos C., Eibl D., Eibl R.. Scale-up of adipose tissue-derived mesenchymal stem cell production in stirred single-use bioreactors under low-serum conditions. *Eng Life Sci.* 2014;14(3):292-303.

6 Jossen V., Kaiser SC., Schirmaier C., Herrmann J., Tappe A., Eibl D., Siehoff A., van den Bos C., Eibl R.. Modification and qualification of a stirred single-use bioreactor for the improved expansion of human mesenchymal stem cells at benchtop scale. *Pharm Bioprocess.* 2014;2(4):311-322.

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius-stedim.com

USA Toll-Free +1.800.368.7178
Argentina +54.11.4721.0505
Brazil +55.11.4362.8900
Mexico +52.55.5562.1102
UK +44.1372.737159
France +33.442.845600
Italy +39.055.63.40.41
Spain +34.913.586.098
Russian Federation +7.812.327.53.27
Japan +81.3.4331.4300
China +86.21.6878.2300

Publication No.: SBT1027-e170802 · Order No.: 85037-559-47 · Ver. 08 | 2017