Mesenchymal stem cell cultivation in single-use stirred tank bioreactors

PromoCell®

Application note

Mesenchymal stem cells have gained significant attention because of their therapeutic potential for many difficult-to-treat diseases. Here, we describe a protocol for Good Manufacturing Practice (GMP) compliant large-scale production of mesenchymal stem cells in a single-use bioreactor.

Background

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into various cell types, including bone, cartilage, muscle, and blood cells. MSCs have shown great potential for use in regenerative medicine because of their ability to promote tissue repair and reduce inflammation. They have been investigated for therapeutic uses in various conditions, including cardiovascular disease, autoimmune disorders, and neurological disorders. MSCs have also been shown to have immunomodulatory effects, which makes them attractive for treating inflammatory conditions, such as arthritis [1]. As they are found in various tissues throughout the body, including bone marrow, adipose tissue, and umbilical cord tissue, MSCs can be harvested *in vivo* and used for cell therapy and tissue repair applications. For example, adipose tissue-derived human mesenchymal stem cells (hMSC-AT) can be isolated from adipose tissue and developed into different cell types *in vitro* for clinical applications [2, 3] (Fig. 1).

Ensuring the quality and safety of cell culture reagents and using GMP grade media are essential when manufacturing MSCs for therapeutic applications. The use of noncompliant materials can lead to contamination and variability in cell behavior. Quality control measures should be in place to monitor the purity, identity, and quality of the cells throughout the manufacturing process. Additionally, GMP grade media is essential for maintaining consistency in MSC growth and differentiation. Adhering to these standards is key for the effective and safe use of MSCs for clinical use [4, 5].

MSCs are a relatively rare cell population, and large quantities of cells are typically needed for clinical applications. This creates the need for large-scale platforms to efficiently expand MSCs [6, 7, 8]. Bioreactors are useful tools for up-scaling MSC cultures because they provide a controlled environment for cell growth through culturing at optimal temperature, oxygen, and nutrient supply. Bioreactors also offer the ability to monitor and adjust cell behavior in real time, allowing for the large-scale production of MSCs with consistent characteristics [9, 10].

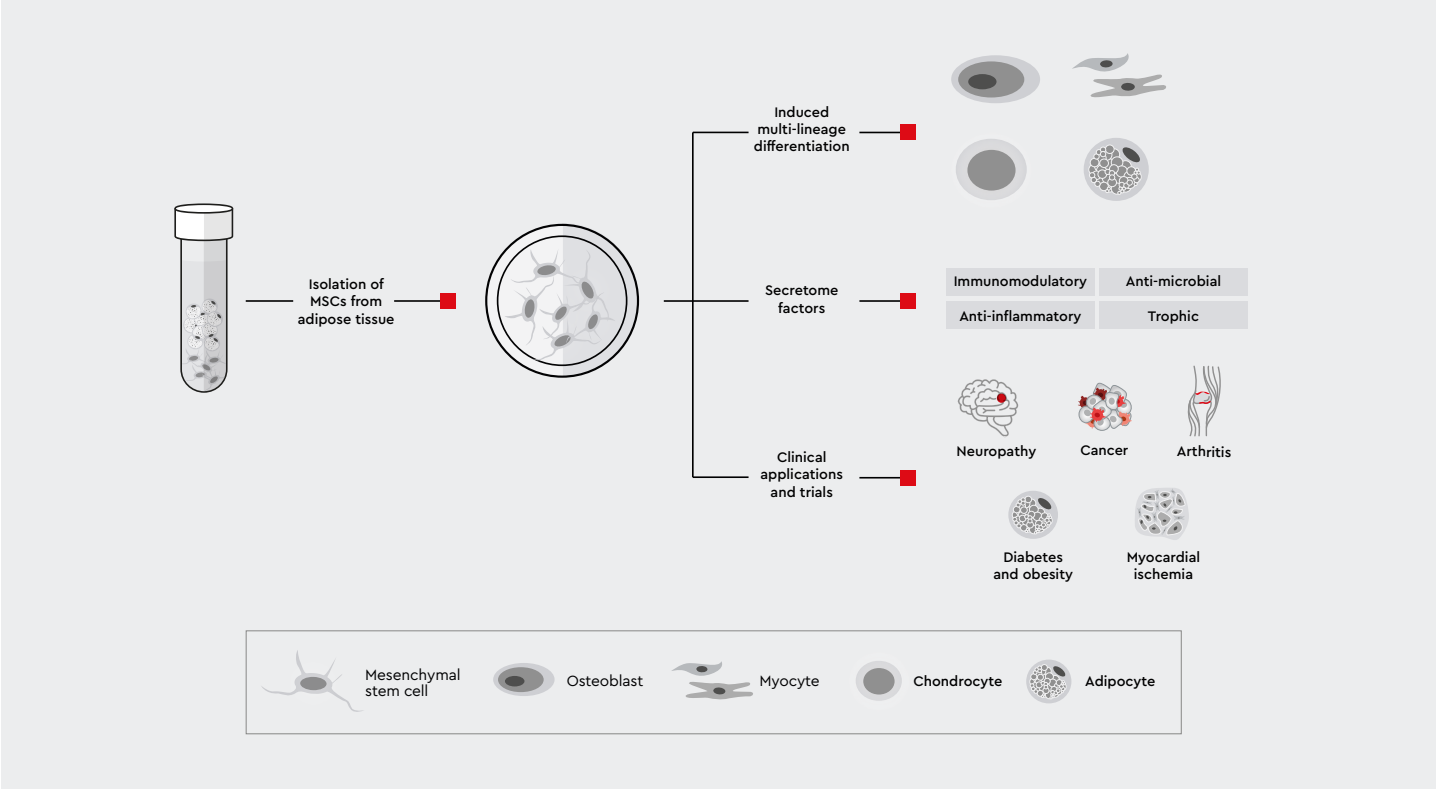

Fig. 1: *Overview of hMSC-AT isolation using density-based centrifugation and the cell-adhering properties of stem cells. After isolation, hMSC-AT can be expanded in vitro for clinical applications.*

Bioreactor MSC expansion protocol

I. Materials

- Adipose tissue-derived human Mesenchymal Stem Cells (hMSC-AT) (C-12977, PromoCell)
- Mesenchymal Stem Cell Growth Medium XF (C-28019, PromoCell)
- Accutase-Solution (C-41310, PromoCell)
- Freezing Medium Cryo-SFM (C-29910, PromoCell)
- Trypan Blue solution 0.4%
- Inverted microscope and cellSens software (Olympus)
- Image analysis cellSens software for determining cell attachment distribution onto microcarriers
- DMCs (Dissolvable MC Synthemax™ II, Corning)
- Single-use AppliFlex ST 500 ml bioreactor with a capped L-type sparger (Getinge) (Fig. 2)

Fig. 2: *AppliFlex ST 500 ml bioreactors for different cell culture applications.*

II. Expansion protocol

1

Thawing of MSCs

T25 and T75 cell culture flasks were pre-coated with fibronectin to promote cell attachment in xeno-free medium. The vial containing hMSC-AT was thawed in a 37°C water bath for 2–3 minutes according to the PromoCell instruction manual. Subsequently, cells were seeded in the coated T25 and T75 flasks, pre-filled with Mesenchymal Stem Cell Growth Medium XF. The seeding density was 4,000 cells per cm^2. Cells were cultured up to a maximum of 4–5passages.

2

Subculturing of MSCs

The culture medium was replaced 4 hours after initial seeding to remove residual freezing medium. Thereafter, the medium was replaced every 3–4 days to replenish glucose and other nutrients. Cell growth was monitored using an inverted microscope and the Olympus cellSens image software. Once 70–90% confluency was reached, the cells were subcultured according to the PromoCell Accutase-Solution instruction manual. Viability was determined manually using the Trypan Blue exclusion test (Trypan Blue solution 0.4%), a Neubauer chamber, and an inverted microscope. Cells were then re-seeded in new T-flasks at the predetermined concentration of 4–8,000 cells per cm^2.

3

Cryopreservation of MSCs

Once cell confluency in the T-flasks reached 80–90%, cells were cryopreserved at a concentration of 2–4 × 10^6 cells per ml in Cryo-SFM in cryo-vials according to the instruction manual.

4

Microcarrier testing

Dissolvable microcarriers (DMCs) and polystyrene microcarriers (PMCs) (Corning Inc. Dissolvable Microcarriers Synthemax II and Corning Inc. Low Concentration Synthemax II Microcarriers) were prepared and tested according to the manufacturer's instructions. Both types of microcarriers were hydrated and resuspended in medium to achieve a final concentration of 0.01 g/ml for DMCs and 0.1 g/ml for PMCs. Two ultra-low attachment 6-well plates (Cat. No. 3471, Corning Inc.) were used to test each microcarrier type and two initial cell concentrations of 4,000 and 10,000 cells per cm^2 surface area. Microcarrier dissolution/detachment (DMC/PMC) was achieved using the respective harvesting solutions per the manufacturer's instructions.

5

Cultivation in the bioreactor

A single-use AppliFlex ST 500 ml bioreactor (Cat. No.: Z655005112 R03, GETINGE), equipped with a three-blade pitched marine impeller was used as a vessel for the growth of hMSC-AT microcarriers. A capped L-type sparger was used instead of a microsparger to limit foam formation during the batch process. A total working volume of 250 ml was added to the bioreactor with dissolvable microcarrier concentrations of 1.6 g/l and 28 g/l, respectively. A total of 8 × 10^6 cells were seeded, corresponding to 4,000 cells per cm^2 microcarrier surface area or 32,000 cells/ml. The bioreactor settings were controlled at 37°C, pH 7.3 (down-control with CO_2), and dissolved oxygen (DO) above 20% (up-control with air).

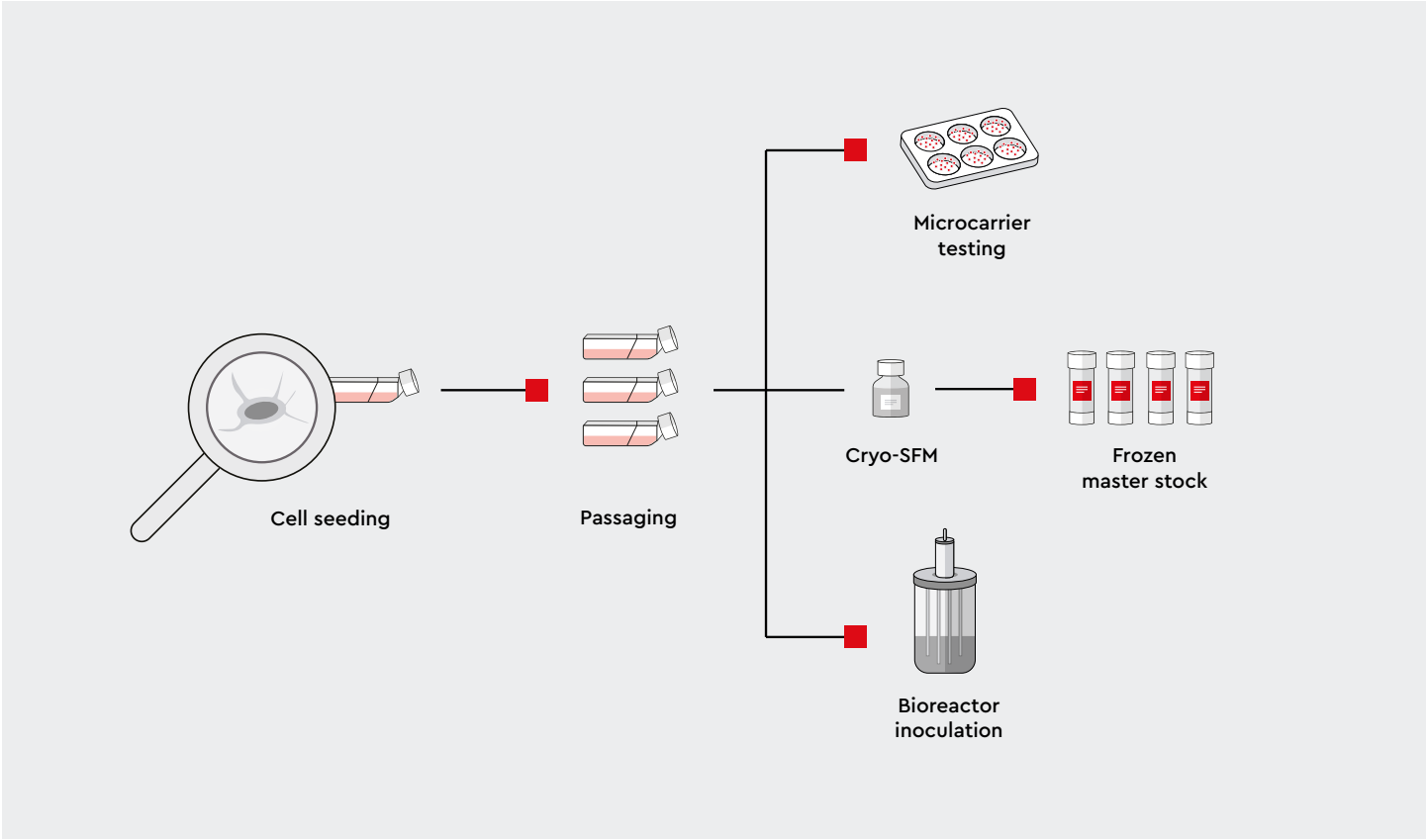

Fig. 3: *Overview of the workflow of stem cell expansion, storage, testing, and bioreactor inoculation.*

Results

I. Attachment and cell expansion

Successful cell-to-microcarrier attachment and distribution in the bioreactor are shown in Fig. 4A and 4B. After 4 hours, 62% of cells were successfully attached. On day one, 87% of the DMCs contained visibly attached cells.

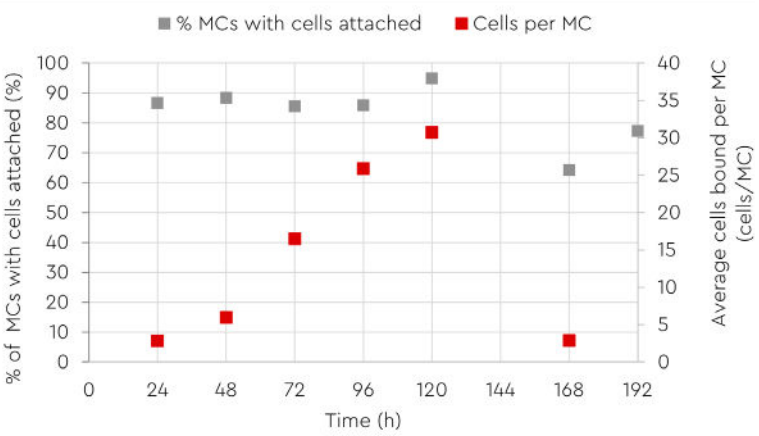

Fig. 4A: *Percentage of microcarriers with attached cells and number of cells per microcarrier.*

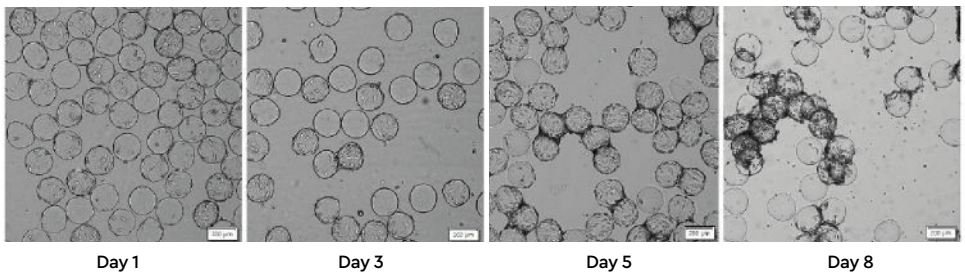

Fig. 4B: *Microscope images of the DMCs with cells attached during 8-day batch run (scale bar: 200 µm).*

During batch culture, a 5.5-fold expansion was achieved, with a maximum measured cell concentration of 1.8×10^5 cells/ml and 97% viability on day 5 of the batch (Fig. 5). The cell doubling time was 30 hours in the bioreactor and 27 hours in planar flasks. The total number of cells measured in the entire batch was 45 million, which is nearly half of the cell number required for clinical applications.

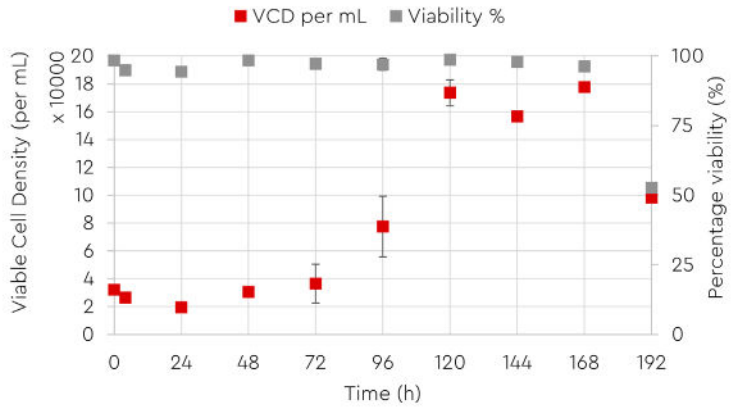

Fig. 5: *Viable cell density and viability (%) of stem cells up to 192 hours.*

II. Metabolite analysis

The levels of glucose, lactate, glutamine, and glutamate in the medium were analyzed over time. Fig. 6 shows increasing lactate concentrations (reaching a maximum of 0.85 g/l) and decreasing glucose concentrations, starting at 0.9 g/l and reaching 0 g/l by day 7.

Although the concentration of glutamine decreased over time, it did not reach 0 g/l by day 8 (Fig. 7), as was observed for glucose (Fig. 6). These results suggest that glutamine is not the limiting factor for further expansion of hMSC-AT in the batch.

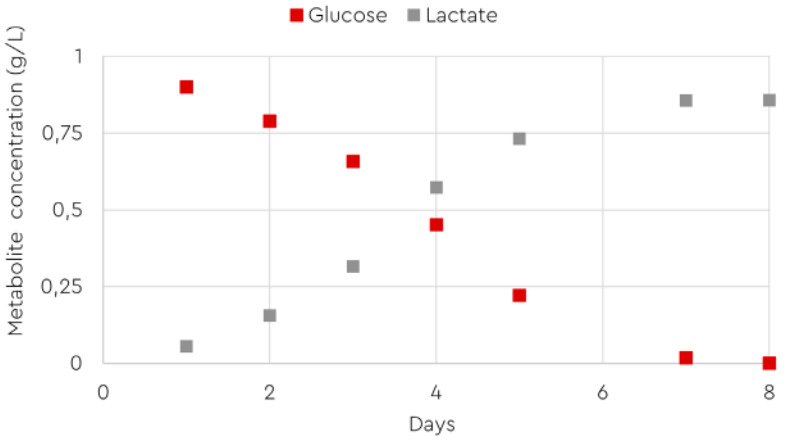

Fig. 6: *Extracellular glucose and lactate concentrations over time.*

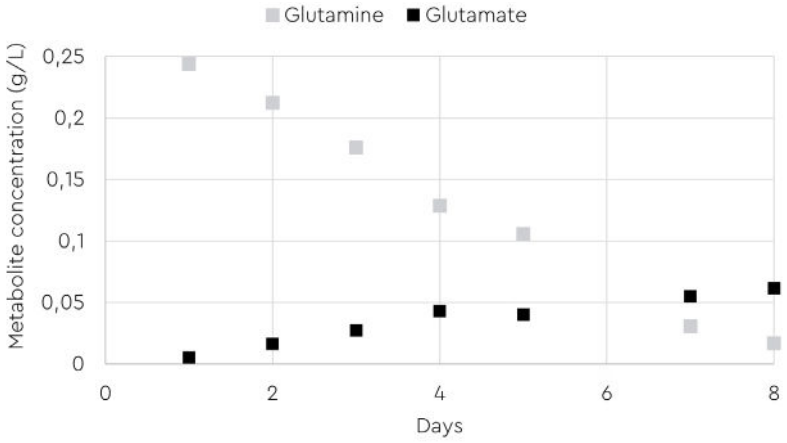

Fig. 7: *Extracellular glutamate and glutamine concentrations over time.*

Conclusion

The successful expansion of hMSC-AT with dissolvable microcarriers in a controlled single-use 0.5-liter bioreactor was achieved using batch-mode operation.

These results are comparable to those obtained with a similar experimental setup in a multiuse bioreactor [10], where up- and down-control of DO was used. The comparison shows that DO up-control alone is suitable for the expansion of hMSC-AT in the bioreactor.

Future experiments are needed to examine the performance of fed-batch operation mode and to test the importance of glucose as a limiting factor. The use of a larger bioreactor volume should also be tested to enable the up-scaling of hMSC cultures.

Meet the expert

The Application Support team from Getinge is composed of scientists with the function of Cultivation Specialist that educate, train, and support users related to applications of bioreactor systems. This support is generated based on practical experiments, collecting and presenting data with regard to the existing product range, in order to respond adequately to the current and future wishes of customers. For any question related to the experiments performed in this Application Note please contact applicationsupport.applikon@getinge.com

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