EFFICIENT EXPANSION OF MESENCHYMAL STEM CELLS IN A CLOSED BIOREACTOR SYSTEM

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ABSTRACT

Cell therapies typically require hundreds of millions of cells for one application. For mesenchymal stem cells (MSCs), a typical dose is based on 2 million cells/kg body weight. These cells are obtained from donors, but initial cell numbers are extremely low. Therefore, these cell numbers need to be increased dramatically before they can be administered to the patient. Standard flask-based cell culture is extremely inefficient for cell therapy production. Flasks require heavy operator involvement and expensive clean room infrastructure, while increasing risk of contamination and operator-related variability. Process automation using closed bioreactor technology can reduce costs and improve quality of cell therapy products. The Scinus Cell Expansion system is a closed, microcarrier-based bioreactor for reliable and efficient expansion of adherent cells from minimal initial cell numbers to clinically relevant amounts.

The Scinus Cell Expansion system was able to expand , early passage (P1) MSCs directly from cryo storage. One million cryo-preserved MSCs were inoculated in the single-use bioreactor bag of the Scinus Cell Expansion system and cultured for 15-19 days to a maximum of 1.97 billion cells (range 1.43-1.97 billion). The Scinus's volume expansion capability was used to maintain an optimal cell/volume ration. Viability of harvested cells was high (average 95%). MSCs expanded using the Scinus Cell Expansion system met the usual criteria as defined by the International Society for Cellular Therapy (ISCT).

THE SCINUS CELL EXPANSION BIOREACTOR

MSCs were cultured in the Scinus Cell Expansion system, a closed bioreactor system for scalable adherent cell culture (Figure 1). The system consists of a hardware cabinet and a single-use bioreactor bag that is part of a medium-perfusion loop.

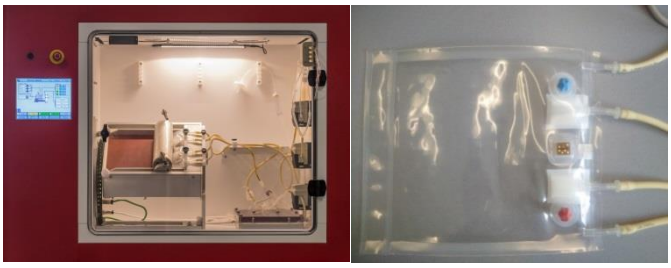

Figure 1 The Scinus Cell Expansion system, consisting of a hardware cabinet enclosure (left) and a single-use bioreactor bag (right)

The single-use bioreactor bag serves at the container for the cultured cells. Inside this bag, MSCs are grown on microcarriers that are retained within the bag by a filter system. This culture method allows continuous cell growth, without the need for passaging (harvest and reseeding). Instead, addition of fresh microcarriers continually increases the available surface for cell growth. Combined with the SCINUS's volume expansion capabilities, this ensures the system's high cell expansion capabilities. The bag contains chemical-optical sensors that detect the dissolved oxygen (DO) concentration and pH. Gas exchange, through an oxygenator that is integrated in the medium perfusion loop, maintains DO and pH at desired set points (pH range 6.0-9.0 and DO 0-100%).

EXPANSION OF CRYO-PRESERVED MSCs

MSCs were isolated from bone marrow of patients undergoing total hip replacement surgery. The mononuclear fraction was obtained after density gradient isolation using Lymphoprep (StemCell Technologies) and expanded for one passage in hMSC-expansion medium in cell culture-treated T-flasks. At 80% confluence cells were harvested and stored in liquid nitrogen (1 million cells/vial) until inoculation in the Scinus Cell Expansion system.

The starting volume of the Scinus single-use bag was set to 80 mL and 5 gram/L of denatured collagen-coated dissolvable microcarriers (Corning Life Sciences) were added, resulting in 2000 cm^2 of

available culture surface. Medium was set to equilibrate to a DO of 75% and a pH of 7.3 before inoculation. One vial of stored cells was retrieved from the liquid nitrogen, thawed and resuspended in circa 10 mL medium. The cell suspension was introduced into the Scinus Cell Expansion system and cells were allowed to attach for 24 hours. Perfusion, as well as pH and DO control was turned off for the seeding phase.

After 24 hours perfusion, pH and DO control were initiated (DO 75%, pH 7.3) and an agitation regime was used to maintain a homogeneous cell suspension. On day 4 and every 2-3 after, a homogeneous sample was taken for visual inspection, cell count, viability and glucose measurements. Fresh medium was added when glucose concentrations fell below a pre-established concentration of 2 mmol/L. Available surface area was increased at two time points to accommodate the growing cell population by increasing the volume of the single-use bioreactor bag and adding additional medium and microcarriers. The final volume was approximately 1300 mL with a total concentration of 5 gram microcarriers/L, representing a total available surface area of 32,500 cm^2, i.e. more than 16x the initially available surface area. The experiment was repeated with three different donors.

RESULTS OF SCINUS-BASED EXPANSION OF CRYO-PRESERVED MSCs

MSCs cultured in the Scinus Cell Expansion system reached a maximum of 1.97 billion cells (range 1.43-1.97 billion) in 19 days of culture (range 15-19 days), see Figure 2. Cell harvest was performed by treatment with an enzymatic detachment agent (TrypLE, Invitrogen) followed by complete dissolution of the dissolvable microcarriers. Cells were recovered from the bag with high efficiency (>85%) and the harvest procedure resulted in a single cell suspension, without presence of microcarrier fragments (Figure 4). Harvested cells had high viability (>95%), as determined by analysis with an NC-250 automated cell analyzer (Chemometec).

MSCs grown in the Scinus Cell Expansion system adhered to criteria for MSCs set forth in the 2006 position paper (Dominici et al., Cytotherapy, 2006). Flow cytometric analysis (MSC analysis kit, BD Biosciences) showed that the cells were positive for CD73, CD90 and CD105 and negative for CD45, CD34, CD11b, CD19 and HLA-DR. Furthermore, cells retained their plastic adherence capability and a spindle shaped morphology. Differentiation along adipogenic and osteogenic lineage was also observed. Chondrogenic differentiation was not successful for both monolayer control and Scinus-expanded, attributable to the high age of donors.

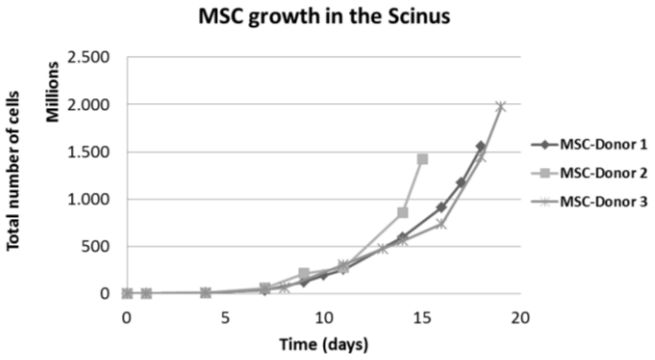

Figure 2 Expansion of cryo-preserved MSCs to over 1 billion cells

	Donor 1	Donor 2	Donor 3
Donor age	71	58	70
Total cell number (millions)	1,560	1,430	1,970
Duration of culture (days)	18	15	19
PDT (hours)	26.8	21.9	28.8
PDL (-)	10.6	10.5	10.9
Viability (%)	N/A	97.0	96.6

Table 1 Characteristics of the three experiments using cryo-preserved MSCs in the Scinus Cell Expansion system

MEDIUM EXPENDITURE

GMP-grade culture medium is a major cost driver for clinical scale production of cell therapies. Expensive components such as (GMP-grade) serum or platelet lysate, and additives such as growth factors, constitute a large part of the total production cost. Minimizing medium expenditure by efficient use of volume-to-surface ratios contributes to keeping cell therapy production affordable. The use of microcarriers in the Scinus Cell Expansion system allows for high-density cell cultures that use minimal amounts of medium. Compared to monolayer culture, with medium refreshments every 2-3 days, the Scinus uses >50% less medium.

BIOMASS MONITORING

Knowledge of the state of a cell culture is important, because the quality of a cell product depends on the timing of the various process steps (e.g. expansion, medium refreshment and cell harvest). Information on the number of cells in culture therefore is crucial, but often not directly available. Operators rely on indirect measurements such as visual inspection or metabolite measurements to determine the time of medium refreshment and cell harvest.

We examined the use of the Scinus Cell Expansion system's online biomass monitoring capacity. The biomass monitoring system measures capacitance, which directly correlates with the membrane-enclosed volume above the sensor. This, in turn, is a direct

measure for the total cell concentration in the measured volume. We determined the predictive power of the capacitance as a measure for cell concentration. We found that capacitance was highly predictive (Figure 3, coefficient of determination, R^2, was 0.989). Consequently, cell concentrations inside the bioreactor bag can be accurately measured without the need for sampling.

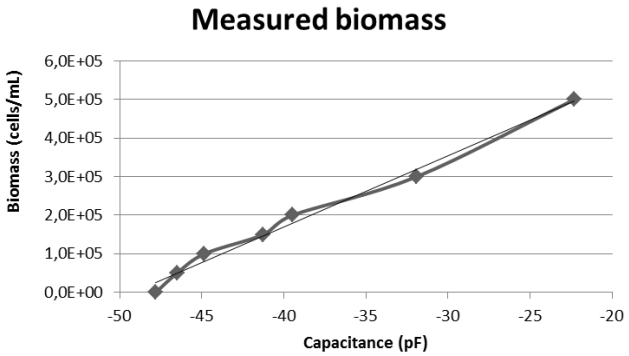

Figure 3 Capacitance, as measured by the integrated biomass sensor, as predictor for cell concentration inside the bioreactor bag. Coefficient of determination, R^2=0.989

CONCLUSION

Results show that MSCs can be efficiently cultured inside the Scinus Cell Expansion system. Starting from one vial of cryo-preserved MSCs, total numbers reached a maximum of 1.97 billion cells (range 1.43-1.97 billion) in little over two weeks. These cells could then be efficiently harvested by completely dissolving the microcarriers, resulting in a single cell suspension with a high viability (>95%).

The use of microcarriers also resulted in minimal medium expenditure. Expansion of one million MSCs to over 1 billion using the Scinus Cell Expansion system cost approximately 50% less medium than the corresponding monolayer culture.

Finally, continuous monitoring and control of the cell culture environment can maintain the perfect conditions for cell growth. Biomass monitoring capacity further allows users to monitor growth without the need to sample.

The Scinus Cell Expansion system can therefore be used to cost-effectively culture MSCs. Large-scale culture of other adherent cell types is potentially also highly cost-effective using this closed bioreactor system.

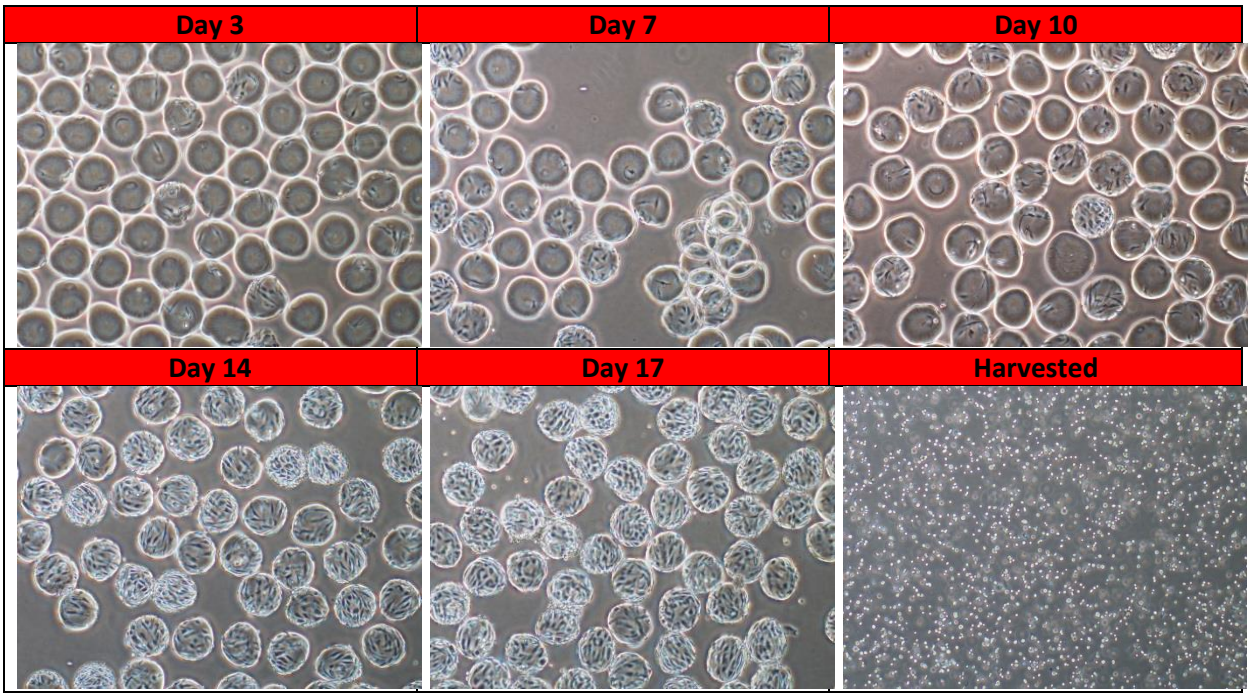

Figure 4 Visual inspection of MSC growth inside the Scinus Cell Expansion single use bioreactor bag. Fully confluent microcarriers were observed near the end of culture. Final pane: harvest through completely dissolving the microcarriers resulted in a single cell suspension without the presence of microcarrier fragments.