Large-scale expansion of MSCs using one-step, closed-system bioreactor technology

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INTRODUCTION

Cost-effective production of cellular therapies requires efficient culture platforms that address major cost drivers: labour costs, clean room requirements and consumable expenditure. At the same time, process automation can increase quality and reliability of the cell product.

Therapies using mesenchymal stem cells (MSCs) represent a major part of cell-based clinical trials. Consequently, this cell type serves as an excellent source to demonstrate (cost-) effective culture using bioreactor technology.

We demonstrate efficient large-scale culture of MSCs, using the Scinus Cell Expansion system (figure 1). Minimal starting amounts are grown to high cell numbers in a closed, controlled system that requires minimal operator time and minimizes use of cell culture medium.

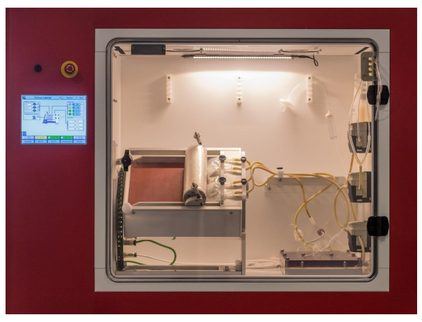

Figure 1 The Scinus Cell Expansion system

MATERIALS AND METHODS

Human MSCs were isolated from bone marrow obtained from patients undergoing total hip replacement surgery. The mononuclear fraction was seeded on cell culture plastic at a density of approximately 100,000 MNC/cm^2. Passage 1 MSCs were harvested after 6-7 days and stored in liN$_2$ until the start of the experiments.

One-step expansion of cryo-preserved MSCs

A Scinus Cell Expansion system was prepared using the following initial conditions

Parameter	Value
Volume	80 mL
Microcarrier concentration	5 gram/L
Dissolved oxygen (approx. pO$_2$)	75% (17%)
pH	7.3
Temperature	37 ^{o}C
Inoculation	1 million MSCs

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 which contained 80 mL medium and 5 gram/L dissolvable microcarriers (Corning Life Sciences).

Expansion procedure

An agitation regime was used to maintain a homogeneous cell suspension and medium perfusion was initiated to maintain environmental control. A homogeneous sample was taken every 1-3 days for visual inspection, cell count, viability and glucose measurements. Fresh medium was added when glucose concentrations fell below a pre-established concentration. Available surface area was increased to accommodate the growing cell population. The final volume was ±1300 mL (surface area 32,500 cm^2).

Biomass sensor

The Scinus Cell Expansion system is equipped with online biomass monitoring capability. This feature eliminates the need for offline sampling to determine the progress of the culture and to establish the correct day for harvest. The capacitance-based biomass sensor measures a membrane-enclosed volume as a measure for cell concentration. We determined the correlation between measured capacitance (in pF) with offline established cell concentrations.

RESULTS

>10 population doublings within one system

One million cryo-preserved cells were expanded to a total of up to 2 billion cells (range 1.43-1.97 billion, figure 2), without passaging, using the Scinus's volume expansion capabilities. This equates to 10.5-10.9 population doublings. The numbers were obtained 15-19 days after inoculation of one vial of cryo-preserved MSCs.

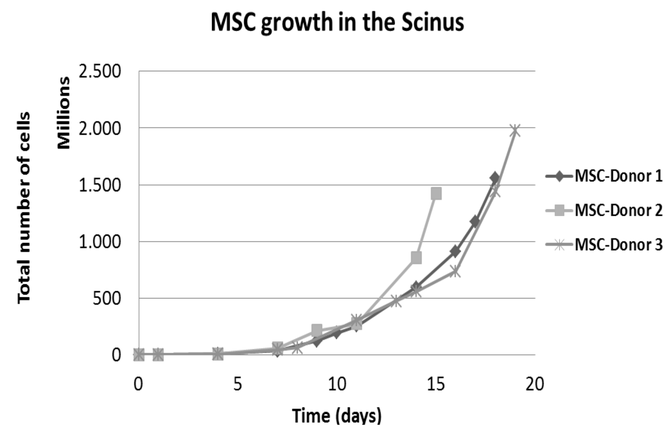

Figure 2 MSCs were cultured using the Scinus Cell Expansion system, to a total of 2 billion

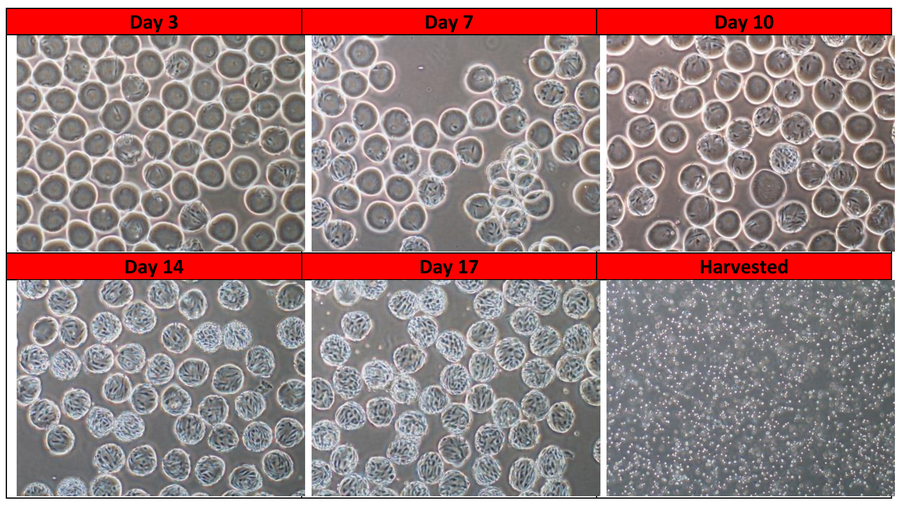

Figure 3 Visual inspection of MSCs on dissolvable microcarriers, sampled from the Scinus Cell Expansion system at different time points

Efficient harvest of high cell numbers

The total cell population was harvested by one operator in one hour. The efficient harvest procedure recovered over 85% of the cells with high viability (>95%). Dissolution of the microcarriers resulted in a single cell suspension after harvest (figure 3).

Parameter	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

Biomass signal and cell density

The online biomass measurements displayed a high correlation between capacitance (in pF) and the calculated biomass (in cells/mL, figure 4). The coefficient of determination, R^2, of the linear regression equation is 0.989. As such, capacitance is an excellent predictor for biomass inside the Scinus system.

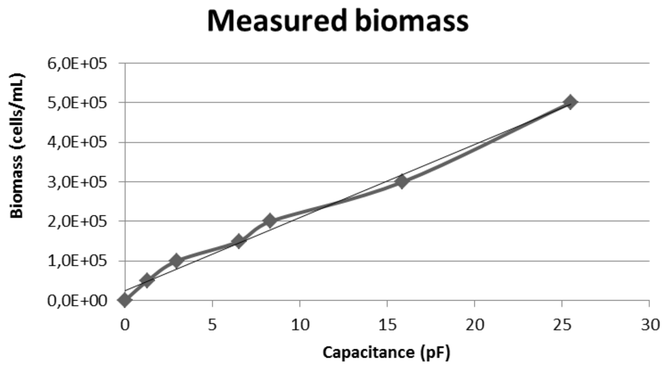

Figure 4 Capacitance, as measured by the integrated biomass sensor, as predictor for cell concentration inside the bioreactor bag. Linear regression coefficient, R^2=0.989

	Operator time	Medium use
Monolayer	22 hr	30 L
Scinus	5.5 hr	13 L
Reduction	75%	57%

Reduction of cost drivers

We calculated operator time and medium usage for the culture of 1 million cells to over one billion. Use of the Scinus Cell Expansion system results in a significant reduction of major cost drivers for cell therapy production.

CONCLUSION AND DISCUSSION

We were able to culture high cell numbers that were not previously obtainable within one closed bioreactor system. Moreover, these high cell numbers were initiated with a small starting population (one vial of cryopreserved MSCs). The closed bioreactor system reduces costs for cell therapy production by lowering operator time, medium expenditure and clean room requirements.

Sampling possibilities and environmental control improve process reliability and product quality. Online biomass measurements give direct information on the number of cultured cells, eliminating the need for sampling.

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