COST-EFFICIENT, CLOSED-SYSTEM MSC CULTURE TO THERAPEUTICALLY RELEVANT QUANTITIES

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INTRODUCTION

Achieving cost-efficient production of cell therapies is a major challenge, with medium costs and operator handling being significant contributors. Medium usage can be greatly reduced by using microcarrier-based expansion to reach high cell numbers in minimal volume. Microcarriers (MC) also enable closed, single-step procedures in bioreactors that limit operator involvement and clean room requirements.

Xpand's Scinus Cell Expansion System is a bioreactor designed for the culture of adherent cells in a closed, single-use bag. This system employs a unique agitation and perfusion approach for efficient MC-based culture processes. Here we present how this system was used to culture cells (MSCs) from pre-culture to clinically relevant numbers with minimal operator involvement, reduced medium usage and efficient harvest. New, denatured collagen dissolvable microcarriers (Corning Life Sciences) were used for their efficient culture, good visualization and easy harvest.

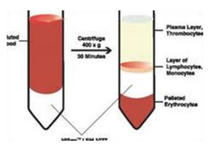
MATERIALS AND METHODS

Pre-culture

BM biopsy

Bone marrow biopsy harvested from donors undergoing total hip replacement surgery

Enrichment



Mononuclear fraction was isolated from the biopsy using density gradient isolation

Passage 0

The mononuclear fraction was seeded at circa 100.000 cells/cm^2 and cultured until confluent colonies appeared

Passage 1

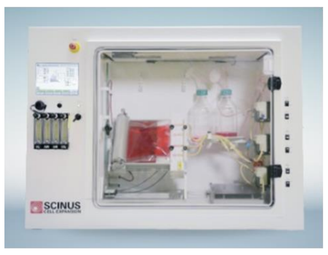
Harvested cells were seeded for a second passage at 400 cells/cm^2 and cultured to subconfluence

SCINUS

A total of **20 million** cells were harvested and seeded in Xpand's Scinus Cell Expansion system. Monolayer cultures were used as control.

Scinus culture

The Scinus Cell Expansion system provided a closed and controlled environment for the duration of the microcarrier-based culture. The system's unique agitation platform maintained a homogeneous culture.



6 days

16% O_2

7.3

37 °C

ca 800 mL

5-10 gram/L dissolvable microcarriers (Corning)

3x/day 1 hour static

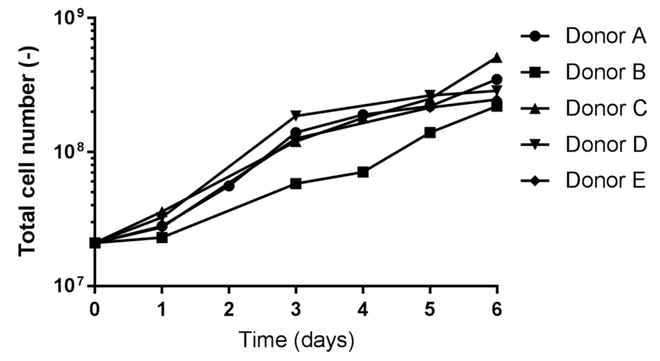
Harvest and analysis

- Cell counts were performed every 1-2 days to determine the growth kinetics during bioreactor culture. Visual inspections were performed for morphology and aggregate formation.
- Cells were harvested by dissolution of the microcarriers inside the bioreactor bag and harvest through the bag's filter outlet. Harvest efficiency was determined based on pre-harvest samples.
- Medium expenditure throughout culture was tracked and compared to monolayer culture to quantify the reduced medium usage.

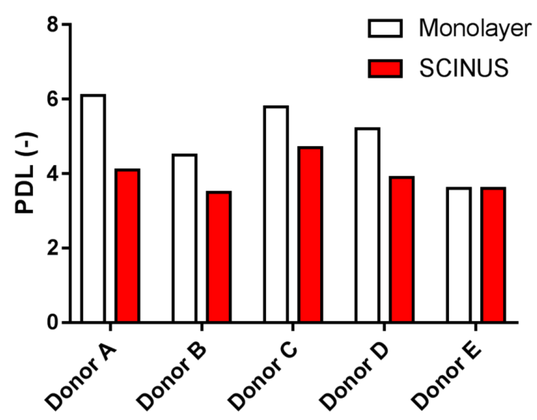
RESULTS

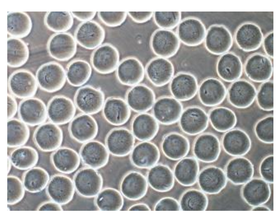
Culture up to 500 million cells in 6 days

Obtaining sufficient cells for clinical applications using efficient culture procedures is a challenge. However, using the Scinus Cell Expansion system a total of 510 million cells were cultured (range 220-510 million) in 6 days. Exponential growth was observed for all donors over the 6 day period. Microcarrier content was increased to 10 gram/L on day 4 to increase the available substrate. The growth in the Scinus Cell Expansion system resulted in an average PDL of 4.0 (monolayer 5.0).

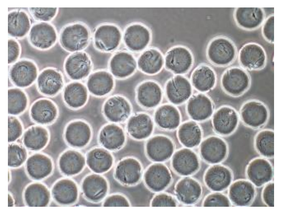


Visual inspection revealed good attachment of MSCs already 2 hours after seeding. Homogeneous coverage was observed on day 4, after which fresh carriers were introduced. Larger aggregate formations were visible after 6 days of culture.

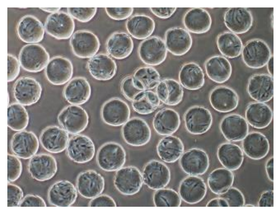




2 hours



Day 1



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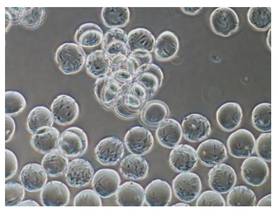
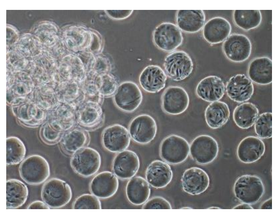


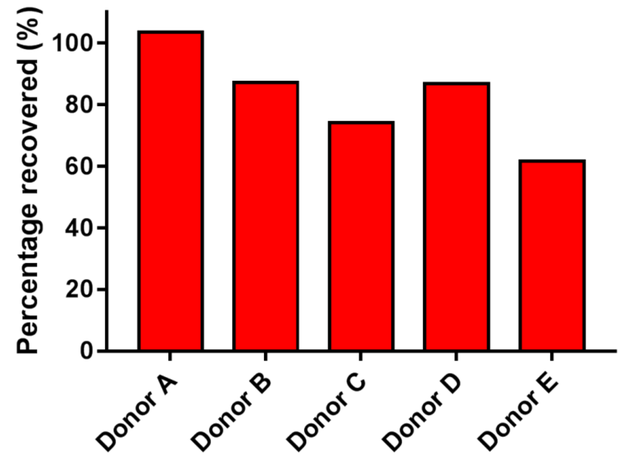
Day 4



Day 6

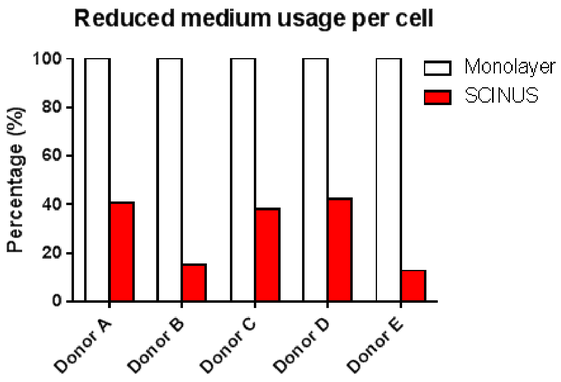
Cells are easily and efficiently harvested

Efficient cell recovery is a challenge when high cell numbers are required. Monolayer cultures can be almost fully recovered, but the process is open, extremely labour intensive and requires multiple operators. Cells in the Scinus Expansion System were efficiently harvested by a single operator in 1.5 hour by complete dissolution of the microcarriers. In a closed procedure, cells were harvested through the bioreactor bag's filter outlet. Average recovery was 82% and with further optimization, >90% is expected. No significant cell death was observed (not shown).



Medium usage is reduced by up to 80%

Medium is a major contributor to the production cost of cell therapies. GMP-grade serum, platelet lysate-based serum replacements as well as expensive growth factors and additive all drive up costs. The efficient surface-to-volume ratio of microcarrier culture resulted in a significant reduction in total medium used. On a per-cell basis the required medium was reduced up to 80% in the Scinus Cell Expansion system.



CONCLUSION AND DISCUSSION

Here we present a cost-effective method of clinical grade MSC culture using a unique bioreactor system with which clinically relevant cell numbers were obtained while reducing production cost. Several hundred million cells were obtained in only 6 days of culture. While the monolayer control culture grew slightly faster (one extra doubling in six days on average), the operator costs are greatly reduced. The production also required significantly less medium than the equivalent monolayer culture would require on a per-cell basis. Since GMP-grade medium contributes significantly to the overall production costs of cell therapies, the Scinus Cell Expansion system can greatly reduce costs. In addition, the closed system, with its scalable culture volume, reduces operator involvement and limits stringent clean room requirements, further reducing production costs of GMP-grade cell products.
The Scinus Cell Expansion system allows the use of different substrates, giving users the opportunity to optimize for their own process. Here we efficiently employed a new, dissolvable microcarrier (Corning) for the production of MSCs.

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