A single-step expansion system for large-fold expansion of bone marrow-derived MSCs

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INTRODUCTION

Cell therapies require (cost-)effective production to ensure that novel therapies are commercially viable. Closed, automated bioreactors can improve handling and safety while also reducing costs by limiting operator involvement, clean room requirements and expenditure of consumables. However, current closed solutions do not support the expansion to hundreds of millions cells from the limited initial cell numbers found in a biopsy without multiple reseeding steps.

We developed novel bioreactor technology (Figure 1) with which high cell numbers can be grown from a bone marrow biopsy in a single expansion system, eliminating the need for labour- and cost-intensive expansion protocols.

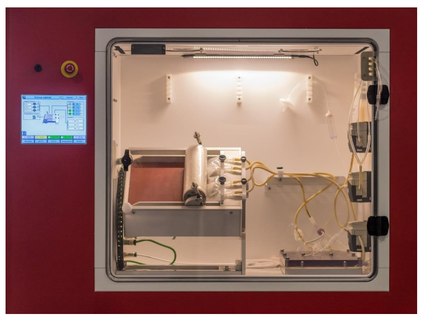

Figure 1 The SCINUS, a bioreactor for large-fold adherent cell expansion

MATERIALS AND METHODS

Biopsies and isolation

Bone marrow biopsies were obtained and processed using Kaneka's CellEffic BM filtration system to enrich for MSCs and reduce RBC content (Table 1).

Table 1 Details of bone marrow biopsies used for inoculation

Parameter	Donor 1	Donor 2	Donor 3
Donor age (years)	32	48	65
Biopsy volume (mL)	25	25	16
Total WBC count (cells)	$270*10^6$	$310*10^6$	$160*10^6$
Viability (%)	98	98	97
Yield after filtration (cells)	$101*10^6$	$75*10^6$	$62*10^6$

Procedure

The resulting heterogeneous cell suspension was introduced directly into a SCINUS. Inoculation was done in 120-135 mL medium on denatured collagen-coated dissolvable microcarriers (Corning Life Sciences). During culture the volume and surface area was increased in two steps to a final volume of 1350 mL with 33750 cm^2 available surface area. Continuous expansion was achieved with bead-to-bead transfer of cells to freshly added microcarriers. The culture environment was controlled at 37 ^oC, pH 7.4 and pO_2 17%. Medium was refreshed whenever [glucose] fell below 2.5 mmol/L. The total procedure is represented in Figure 2.

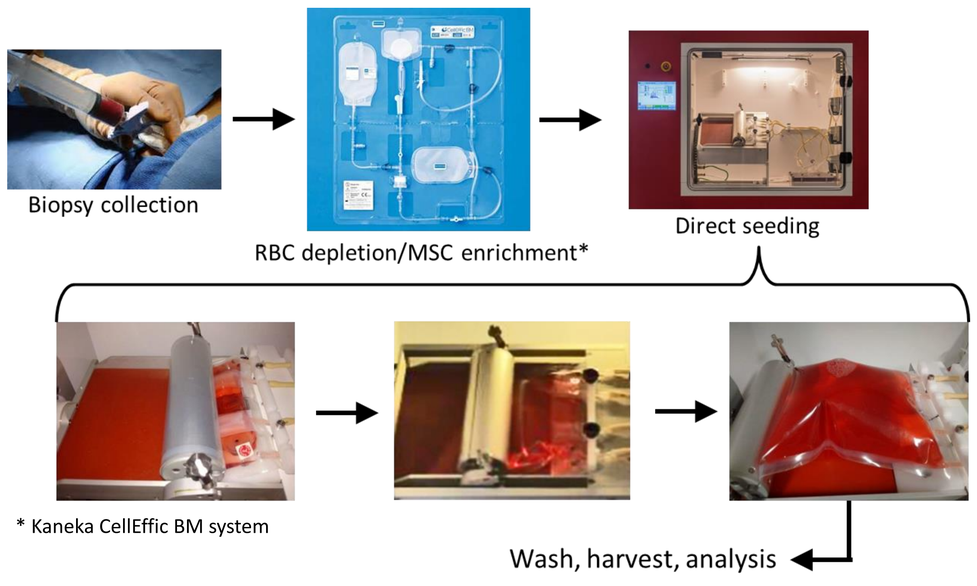

Figure 2 Schematic representation of the expansion cycle of BM-MSCs using the SCINUS

Analyses

Samples were taken every 1-3 days for visual inspection and cell count. Cells were harvested and characterized using flow cytometry (positive markers CD73, CD90, CD105; negative markers CD34, CD11b, CD19, CD45 and HLA-DR).

RESULTS

High cell yield, high viability

Culture was maintained for 19-28 days and over one billion cells (range 1.37-1.73 billion, Figure 3) were obtained. Cell recovery through complete dissolution of the dissolvable microcarriers yielded high recovery rates (average 88%), while maintaining a highly viable population (>97%), see Table 2.

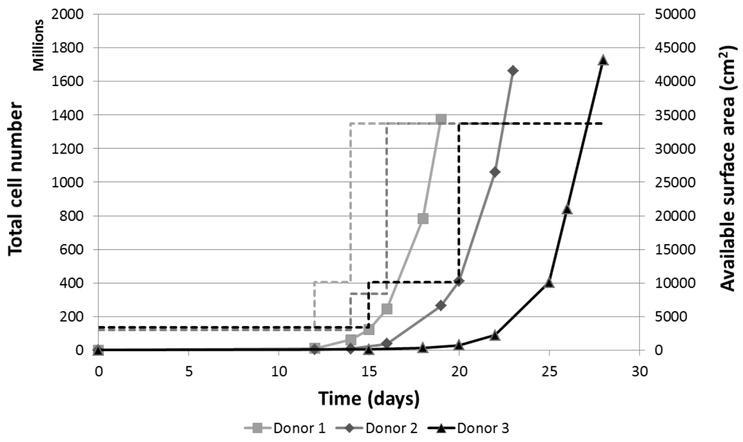

Figure 3 Growth curves of the three donor (left Y-axis) and the corresponding available surface area (right Y-axis)

Low medium expenditure and operator involvement

Total medium usage was low, with only 5.4 L used for the first two donors, while the third donor needed a total of 9.4 L to reach 1.7 billion cells. Hands-on operator time for the total culture procedure was limited. From inoculation to the end of harvest, only 8-10 hours were spent (including sampling and cell count and metabolites)

Table 2 Results of the MSC culture for three different donors. * Including sampling and analysis (cell count and metabolites)

Parameter	Donor 1	Donor 2	Donor 3
Total yield (cells)	$1370*10^6$	$1660*10^6$	$1730*10^6$
PDT (h)	23.6	25.9	36.6
Harvest efficiency (%)	96	80	88
Viability after harvest (%)	97	97	98
Medium used (L)	5.4	5.4	9.4
Estimated total handling time (h) *	8	8	10

Continuous culture through bead-to-bead transfer

Visual inspection of the culture at different time points showed progressive population of the microcarriers (Figure 4). A fully confluent culture was achieved, indicating that bead-to-bead transfer to freshly added microcarriers had occurred.

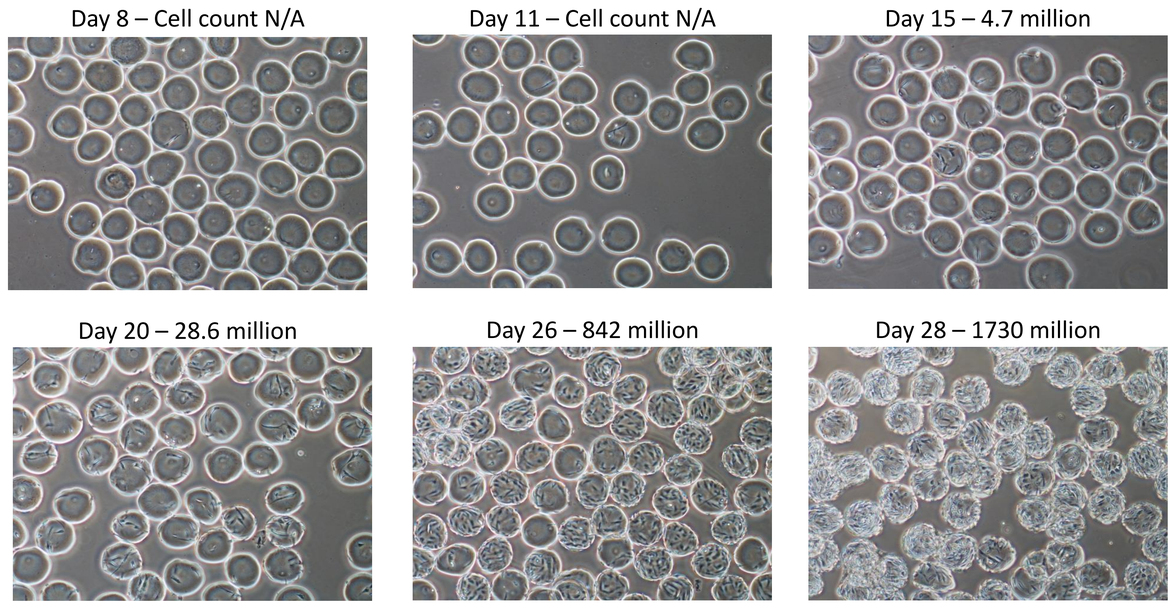

Figure 4 Visual inspection of MSC growth within the SCINUS for donor 3

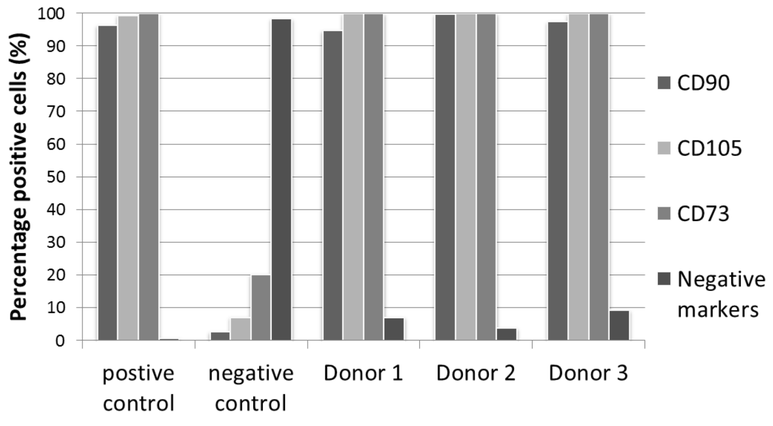

Figure 5 Surface marker expression of harvested cells

Retention of MSC markers

Flow cytometry analysis showed that harvested cells were positive for MSC markers CD73, CD90 and CD105 and negative for markers CD34, CD11b, CD19, CD45, HLA-DR (Figure 5), despite no initial selection on plastic took place.

CONCLUSION AND DISCUSSION

Here we present a method and system that enables manufacturers of cell therapies to achieve high cell numbers from direct inoculation of a bone marrow biopsy. Compared to monolayer culture, this approach significantly reduced required operator time and medium usage, while MSC phenotype is maintained. Passaging of cells was not required, continuous expansion was possible due to cells migrating to freshly added microcarriers. This approach allows manufacturers cell therapies to safely and (cost-) effectively produce therapeutic products.

Acknowledgements: The research leading to these results has received funding from the European Union Horizon 2020 Programme [*H2020/2014-2020*] under *grant agreement* n° [731377]. Kaneka Pharma Europe NV for providing the CellEffic BM devices.

MUS.I.C. Multisystem Cell Therapy for Improvement of Urinary Continence