ONE-STEP BONE MARROW-DERIVED MSC CULTURE USING NOVEL BIOREACTOR TECHNOLOGY

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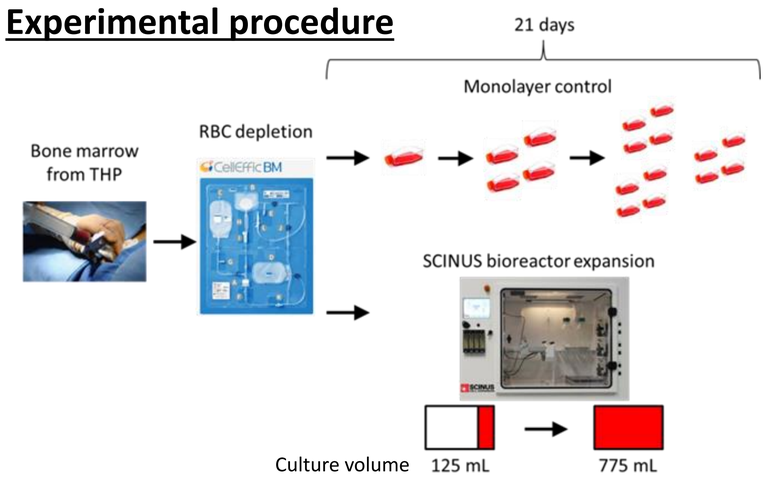
INTRODUCTION

Culture of bone marrow-derived MSCs for clinical application is a costly process, in large part due to the requirement for cleanroom facilities necessitated by numerous open procedures. The use of closed bioreactor systems can reduce costs and improve quality of the final cell product. However, these systems are usually ill suited to culture cells to large quantities directly from an aspirate.

Here we present a closed system to culture millions of MSCs starting from a small volume bone marrow aspirate, while retaining MSC properties.

MATERIALS AND METHODS

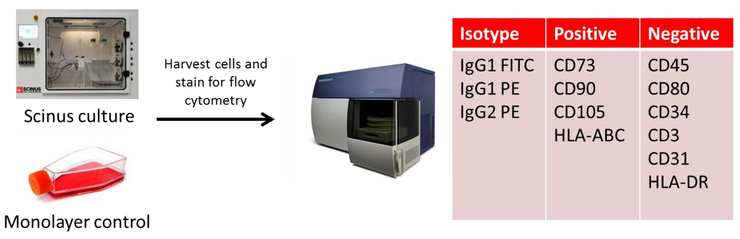
Experimental procedure



A 8 mL bone marrow aspirate was enriched for MSCs using Kaneka's CellEffic-BM filtration system. The resulting cell suspension was seeded in 125 mL in Xpand's Scinus Cell Expansion system (7/8 volume), or cultured on monolayer (1/8 volume). The pH (7.3) and pO_2 (16%) were maintained throughout culture and cell counts were performed every 2-4 days. Medium and microcarriers (Enhanced Attachment, Corning Life Sciences) were added and volume was expanded based on cell and glucose measurements. The final volume was 800 mL.

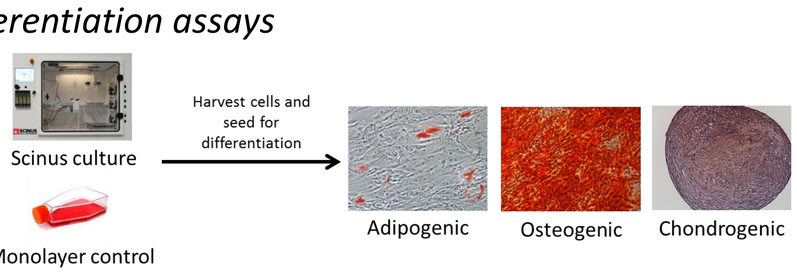
Analyses

Flow cytometry

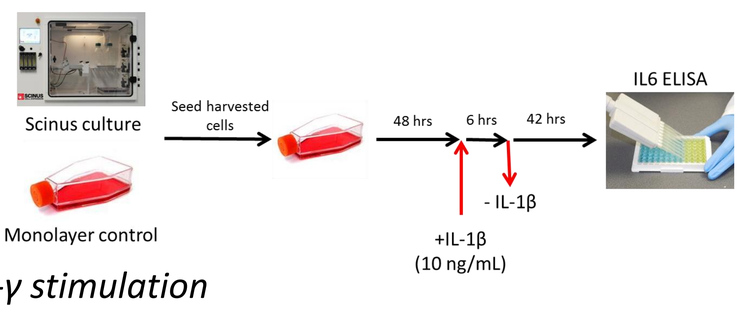


Isotype	Positive	Negative
IgG1 FITC IgG1 PE IgG2 PE	CD73 CD90 CD105 HLA-ABC	CD45 CD80 CD34 CD3 CD31 HLA-DR

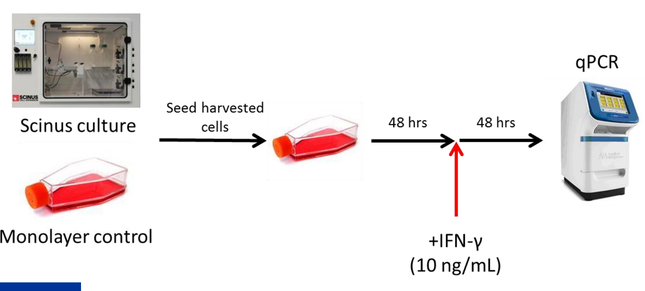
Differentiation assays



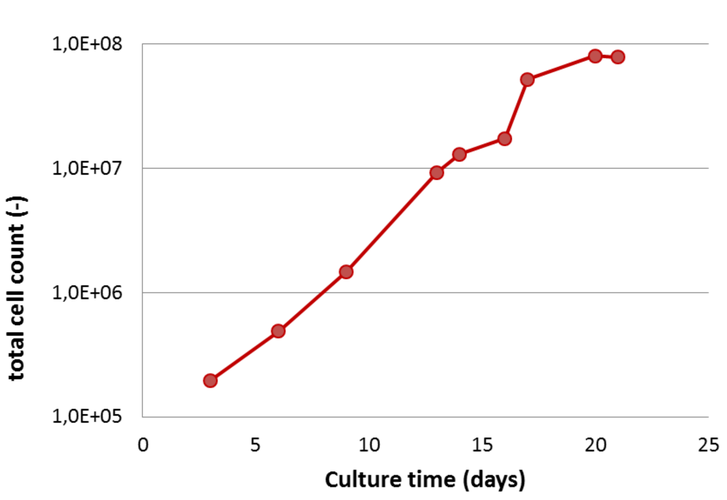
IL-1β stimulation



IFN-γ stimulation

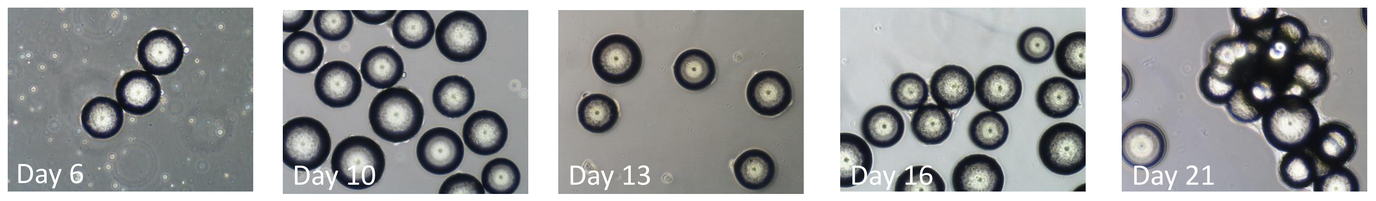


RESULTS



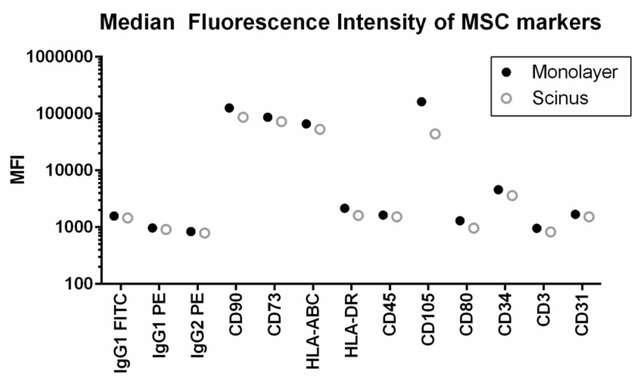
80 million MSCs from 7 mL BM aspirate

A total of $88*10^6$ nucleated cells, theoretically containing 1000-10000 MSCs, were seeded in the Scinus Cell Expansion system. After 21 days of culture a total of 80 million MSCs were harvested from the single use bioreactor bag. Larger aggregates started forming at the end of culture (see below). For the period of day 3 – 20, the PDL was 8.7.



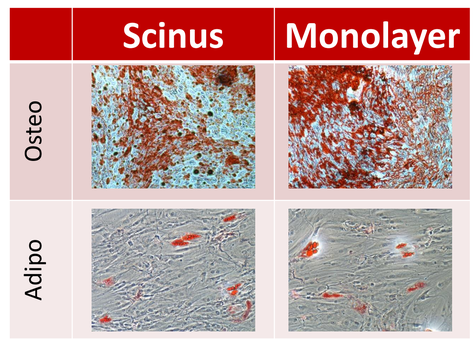
Scinus-cultured MSCs have the correct phenotype

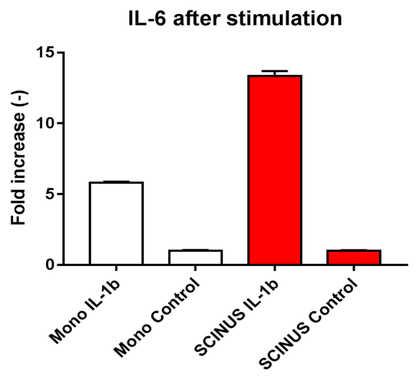
Cell surface markers typically not expressed by BM-MSC (CD3, CD31, CD34, CD45, CD80 and HLA-DR) were clearly absent in both monolayer- and biorector-cultured MSC. Markers typically expressed by BM-MSC (CD73, CD90 and CD105) were present on the MSC populations cultured by either method. CD105 expression was slightly lower in the bioreactor-cultured cells.



Differentiation potential is unaffected by culture method

The differentiation potential of MSCs expanded using either the Scinus Cell Expansion system or monolayer culture was comparable. MSCs successfully differentiated along the osteogenic and adipogenic lineage after stimulation with the respective media. Chondrogenic differentiation was unsuccessful for both methods.



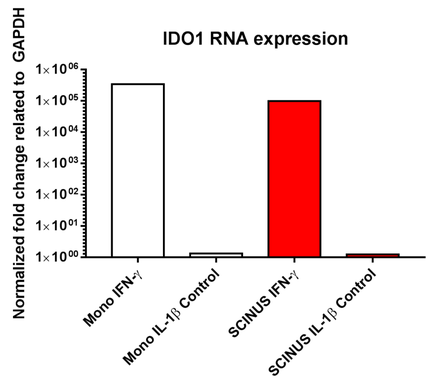


IL-6 is upregulated after stimulation with IL-1β

IL-1b stimulation for six hours resulted in upregulation of IL-6 secretion in both culture conditions. The basal level of IL-6 secretion was higher in the monolayer cultured cells, but the fold increase in response to IL-1β stimulation was higher in the bioreactor-cultured MSCs.

Upregulation of IDO gene expression in both cultures

Stimulation of harvested cells with IFN-γ for 48 hours resulted in upregulation of IDO gene expression in both culture conditions. Stimulation resulted in fold increase of approximately 10^5. Stimulation with IL-1β was used to assess the specificity of the upregulation. Upregulation of IDO and IL-6 after stimulation indicate that the SCINUS culture approach retains the function of MSCs.



CONCLUSION AND DISCUSSION

The SCINUS Cell Expansion system represents closed, controlled bioreactor system for the culture of MSCs directly from aspirate to clinically relevant cell numbers with retention of MSC phenotype and therapeutic potential. The microcarrier-based culture approach eliminates the need to labour intensive passaging steps and the efficient surface:volume ratio minimizes medium requirements. The unique expandable volume of the single use bioreactor bag allows culture initiation with minimum starting volumes, as is evidenced by the successful culture of 7 mL of bone marrow biopsies to 80 million MSCs.

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