CONTROLLED CULTURE OF ADHERENT (STEM) CELLS IN A NOVEL, CLOSED, SINGLE USE BIOREACTOR SYSTEM FOR CELL THERAPY PRODUCTION

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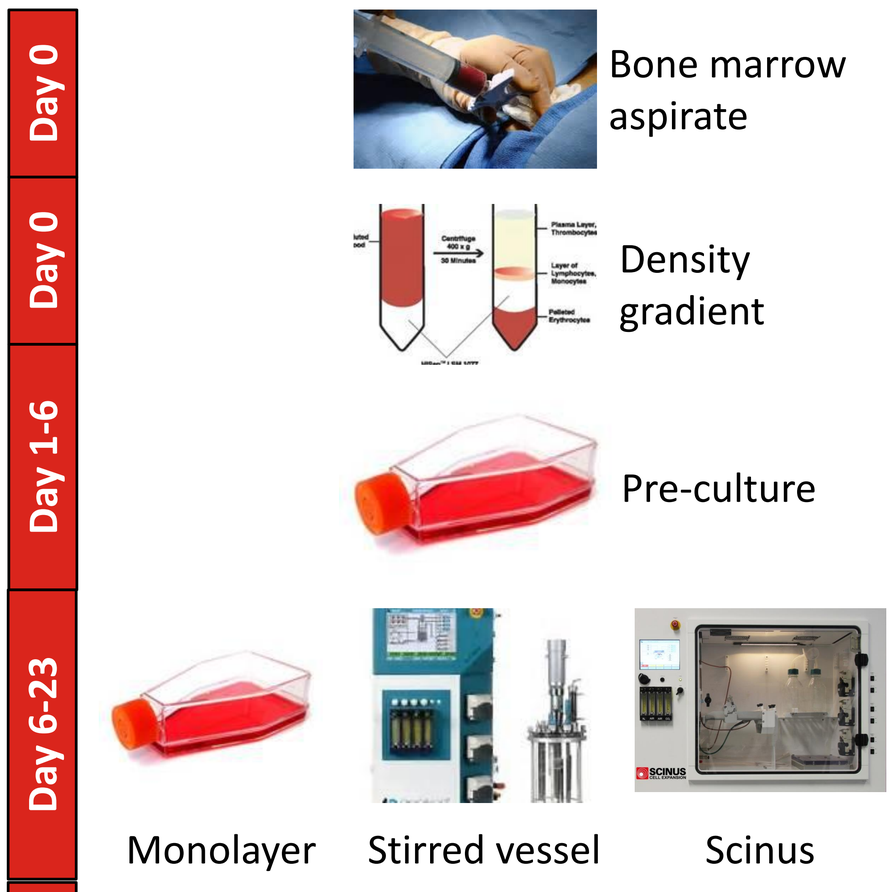
INTRODUCTION

There is an increasing need to make cell expansion cheaper, safer and easier. Standard culture practices should be translated to closed, controlled systems to increase safety and quality. Current closed systems are limited in their culture range, only small maximal cell numbers can be obtained, or the minimal volume is too large to support growth of small cell numbers. Therefore, a bioreactor was designed that supports growth of limited cell number in a minimal volume and allows expansion to therapeutic quantities.

Figure 1 The Scinus Cell Expansion system

MATERIALS AND METHODS

BM-MSCs (P2) were expanded under controlled conditions (DO 25%, pH 7.3) in a closed bioreactor system (figure 1). MSCs were grown for 17 days on microcarriers in an expandable culture bag and compared to stirred vessel and monolayer culture.



MSC response to inflammatory cytokines was assessed. Interferon-γ (IFNγ) treatment stimulated expression of IDO (PCR) and HLA-DR (flow cytometry). Interleukin-1β (IL-1β) exposure stimulated IL-6 secretion (ELISA).

RESULTS

MSC culture was maintained for 17 days (visual inspection, figure 3) in the Scinus system using closed procedures. Volume was expanded twice, resulting in a final volume of 750 mL and 400*10^6 cells (figure 4). Stirred vessel culture, using an identical expansion strategy, yielded 60*10^6 cells (PDL 6.8 vs 4.1).

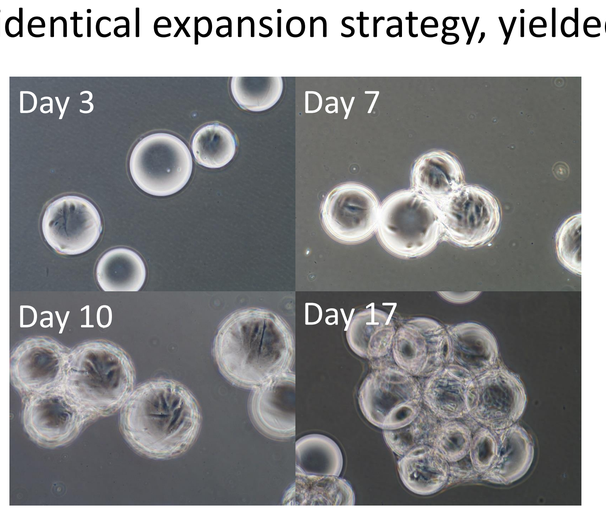


Figure 3 Visual inspection of Scinus-cultured MSCs

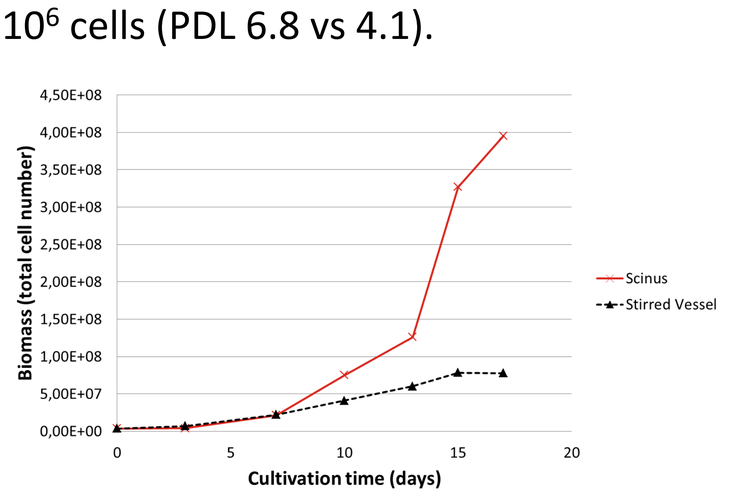


Figure 4 Growth curves of Scinus and stirred-vessel cultured MSCs

All cells were positive (>95%) for CD73, CD90 and CD105 and negative (<2%) for CD43, CD11b, CD19, CD45 and HLA-DR (figure 5). In addition, cells could be differentiated along the osteo- and adipogenic lineage, as assessed using Alizarin Red and Oil Red O stainings respectively (figure 6).

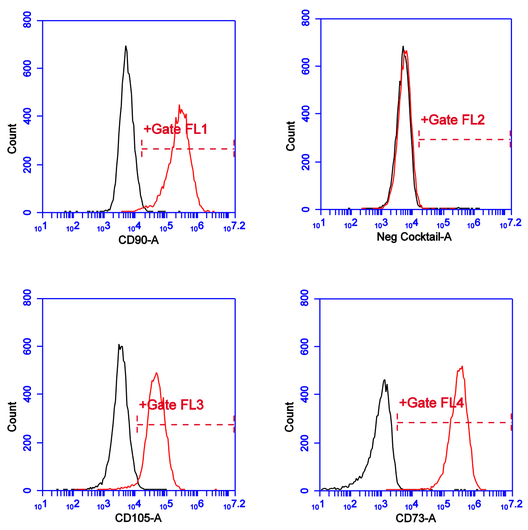


Figure 5 Representative flow cytometry data of Scinus cultured cells. Top left CD90, bottom left CD105, bottom right CD73 and top right negative cocktail (CD34, CD45, CD11b, CD19 and HLA-DR)

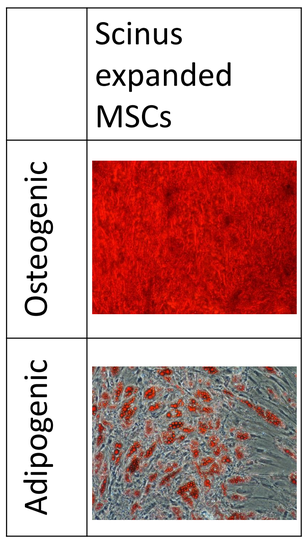


Figure 6 Ostegenic differentiation (Alizarin red) and adipogenic differentiation (Oil Red O) of Scinus-cultured MSCs

Functionality of bioreactor-cultured MSCs was demonstrated using cytokine stimulation (IL-1β and IFNγ). Upregulation of all evaluated markers was comparable between the different culture methods (monolayer, stirred vessel and Scinus, figure 7) after stimulation and for unstimulated (unstim) controls.

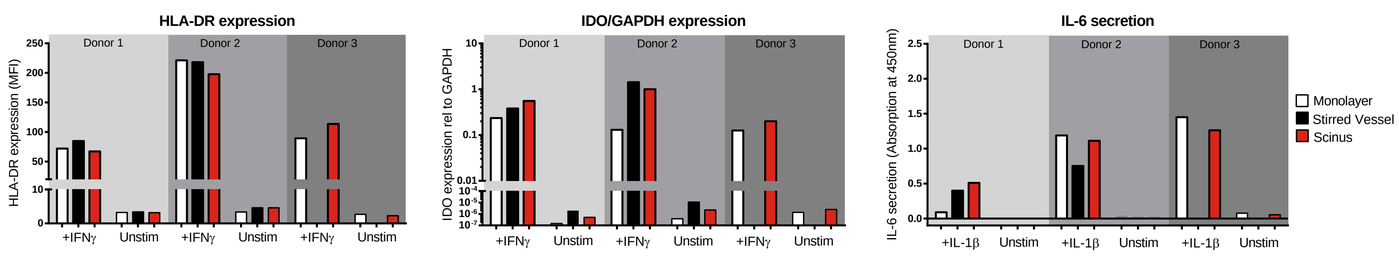


Figure 7 Response to inflammatory cytokines for three different donors. IFN-γ stimulation caused upregulation of HLA-DR expression (left) and IDO gene expression (middle). Stimulation with IL-1β resulted in increased IL-6 protein release (right). Upregulation was comparable between monomalyer, stirred vessel and Scinus cultured MSCs.

CONCLUSION AND DISCUSSION

Successful culture of MSCs in a closed, controlled bioreactor is presented. Scinus-cultured MSCs are morphologically similar to monolayer culture and proliferate faster than in stirred vessels.

Functional potency of the bioreactor-cultured cells was demonstrated using three cytokine stimulation assays. Results indicate that there are no potency differences between the different culture approaches.

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