Xeno-free cultivation of WJ-MSCs in the SCINUS bioreactor

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INTRODUCTION

Wharton Jelly-derived Mesenchymal Stem Cells (WJ-MSCs) have emerged as a promising therapeutic agent for Cell Therapy due to their immunomodulatory and anti-inflammatory properties. In addition, they are not burdened by ethical issues and have minimal risk of malignant transformation [1]. WJ-MSCs are therefore great promise for treating a vast number of difficult-to-treat diseases, such as diabetes, Alzheimer's and ALS. The current limitation is the upscaling of WJ-MSCs to reach sufficient cell numbers for cell therapy. In this study, NextCell Pharma and Scinus Cell Expansion have worked on the upscaling the expansion of WJ-MSCs with an automated bioreactor technology and the validation of their functionality.

MATERIALS AND METHODS

WJ-MSCs from three different donors, were seeded into a SCINUS bioreactor at a cell density of 2200 cells/cm^2 onto Synthemax-II dissolvable microcarriers (SM2-DMC). Cells were cultured in serum-free and xeno-free medium. WJ-MSCs were cultured at 37 °C, with dissolved oxygen setpoint at 75% and a pH of 7.3. Cells were harvested by dissolving the SM2-DMC in the SCINUS bioreactor after 9 to 16 days. During culture, viable cell number was tracked, and cells were tested on functionality.

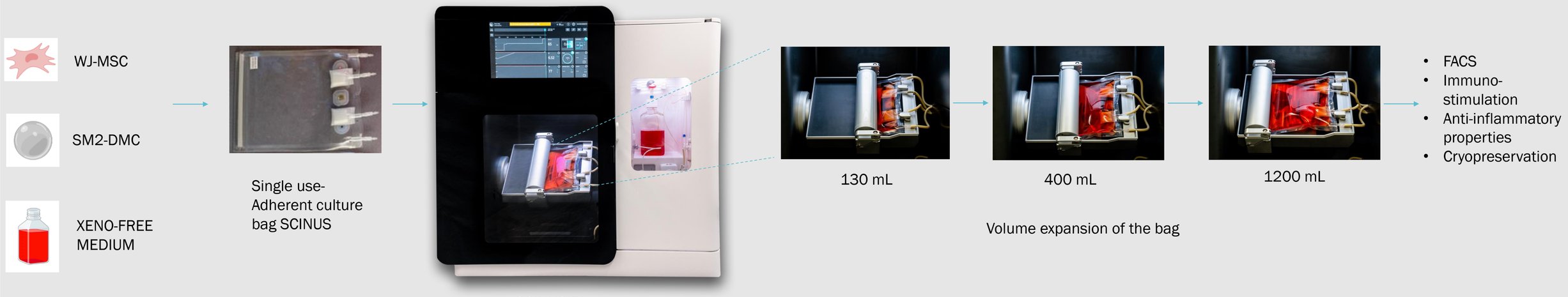

Figure 1 – Workflow of WJ-MSCs in the SCINUS bioreactor

RESULTS

TOTAL CELL NUMBERS

WJ-MSCs were cultured for 9 to 16 days in the SCINUS bioreactor, reaching a viable cell number of 500 million cells or higher. At harvest, all donors had a viability of 95% ± 1%.

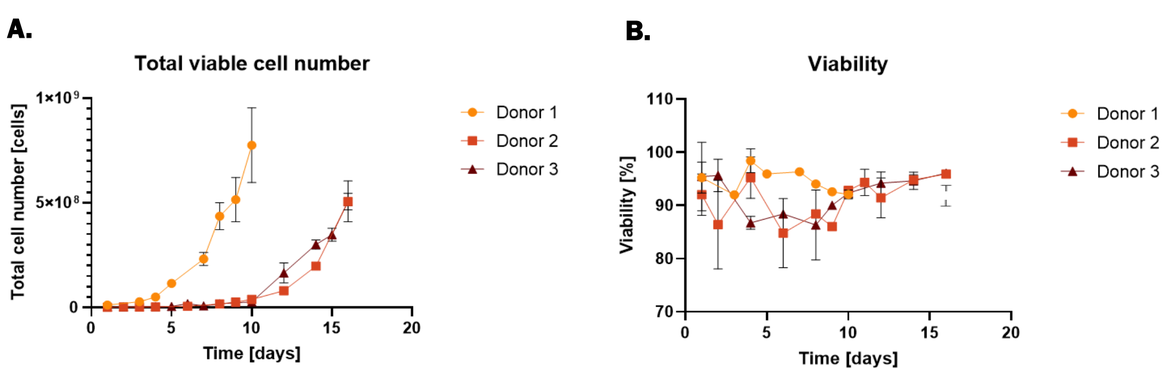

Figure 2 – A: viable cell number of all donors. B: Viability of cells. Cultured in the SCINUS Bioreactor.

Visual inspection revealed that cells retained their fibroblast-like morphology throughout the culture on microcarriers for all donors.

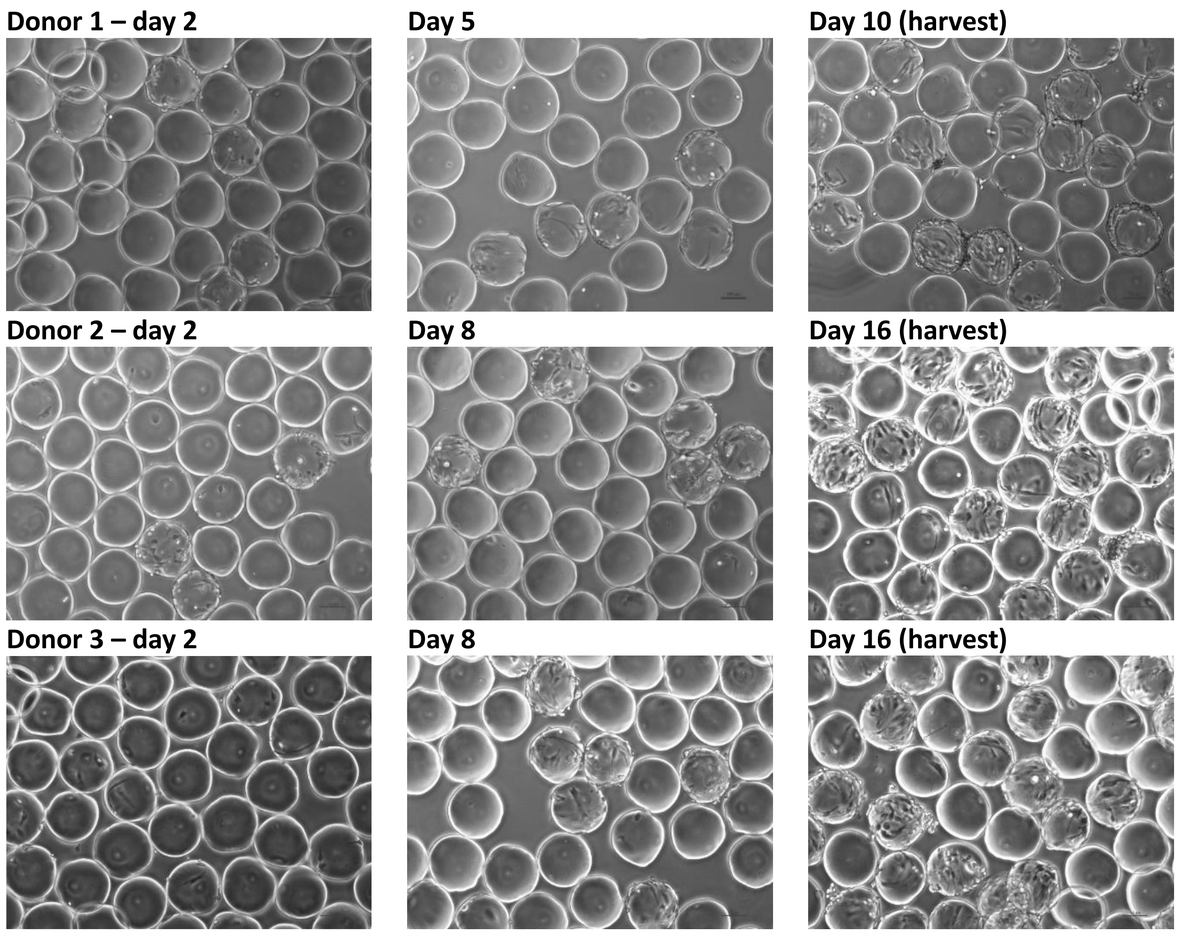

Figure 3 – Visual inspections of WJ-MSCs onto SM2-DMC throughout culture, 40x magnification.

WJ-MSC CHARACTERISATION AND FUNCTIONALITY

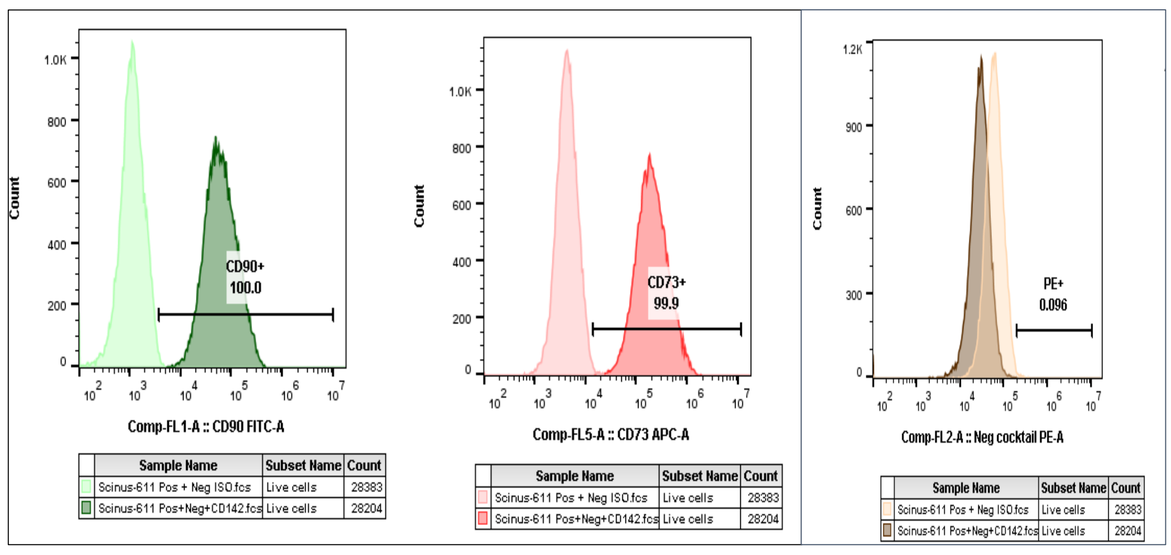

Figure 3 – SCINUS-expanded WJ-MSCs express MSC markers CD90 and CD73 and are negative for CD14, CD11b, CD19, CD45 and HLA-DR

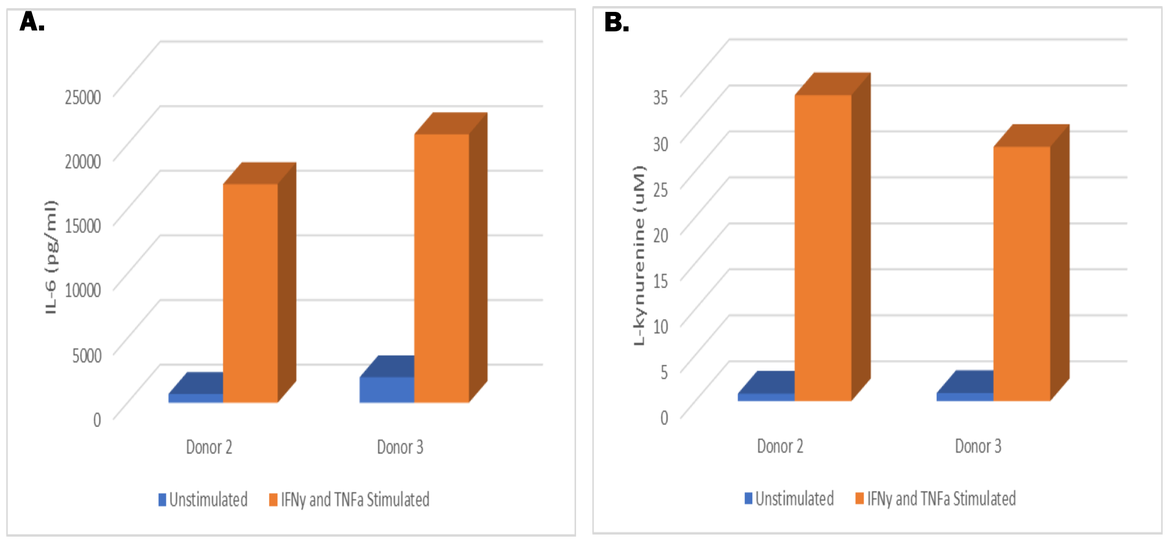

Figure 4 – SCINUS-expanded WJ-MSCs secrete immunomodulatory factors A: interleukin (IL)-6 and B: indoleamine 2,3 dioxygenase (as assessed by measurement of L-kynurenine) in response to stimulation with interferon γ (IFNγ) and tumor necrosis factor α (TNFa).

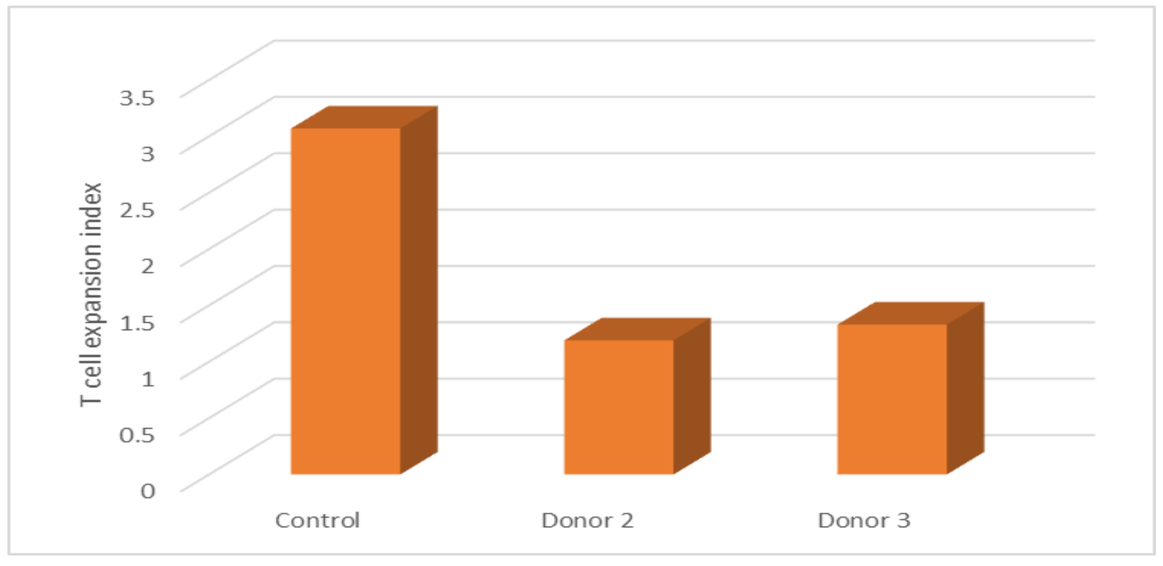

Figure 5 – SCINUS-expanded WJ-MSCs suppress the proliferation of phytohemagluttinin (PHA) stimulated peripheral blood mononuclear cells (PBMCs) labelled with Cell Trace Violet. Expansion index of T cells was measured, and these are defined as CD3+ population. Control represents PBMCs stimulated with PHA in the absence of MSCs.

CONCLUDING REMARKS

In conclusion, we cultured the WJ-MSCs successfully in the SCINUS bioreactor with xeno-free conditions reaching a cell number of at least 500 million cells. Cells retained their fibroblast-like morphology and expressed MSC markers CD90 and CD73. Thereby, the WJ-MSCs kept their immunomodulatory and anti-inflammatory properties. This makes it possible to use WJ-MSCs cultured in the SCINUS bioreactor for cell therapies.

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