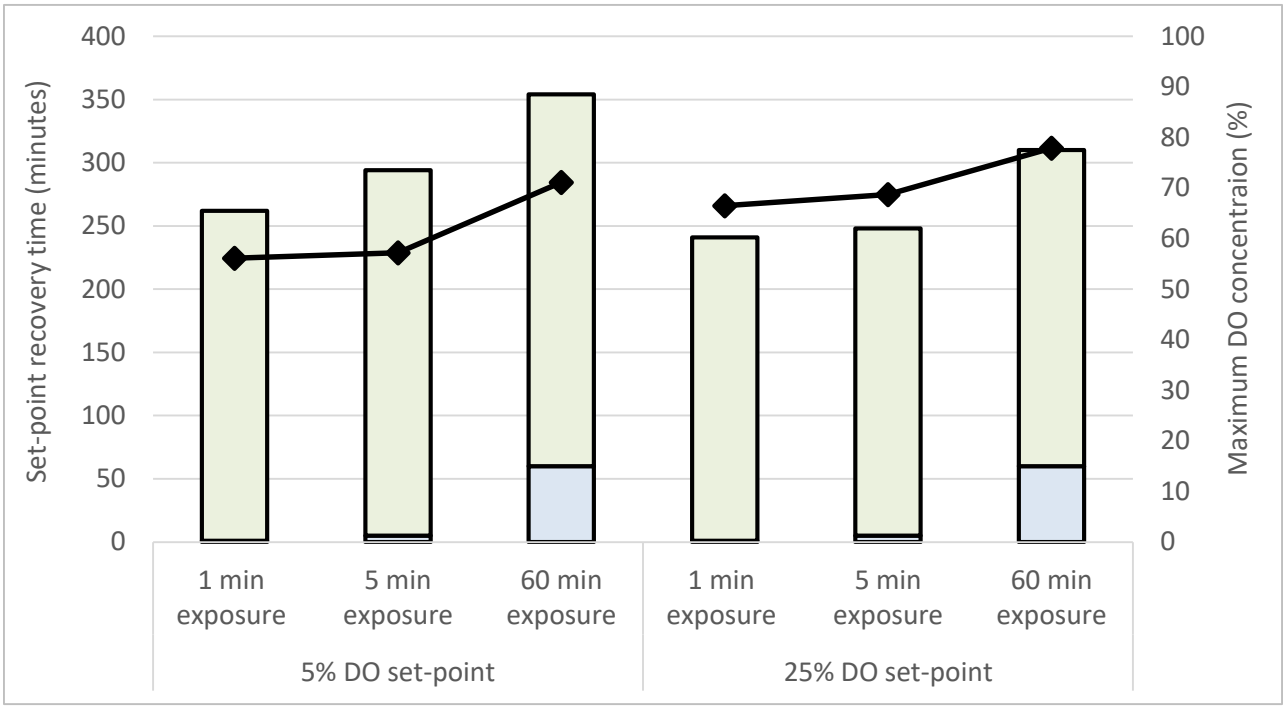


Figure 1 – Implications of exposing low dissolved oxygen (DO) experiments (5 and 25%) to atmospheric conditions in a biological safety cabinet for 1, 5 and 60 minutes. Demonstrating the importance of continuous closed process control during bioreactor culture. Bars represent the set-point recovery time to 5 or 25% DO and diamonds represent the maximum DO concentration reached during each exposure and recovery period.

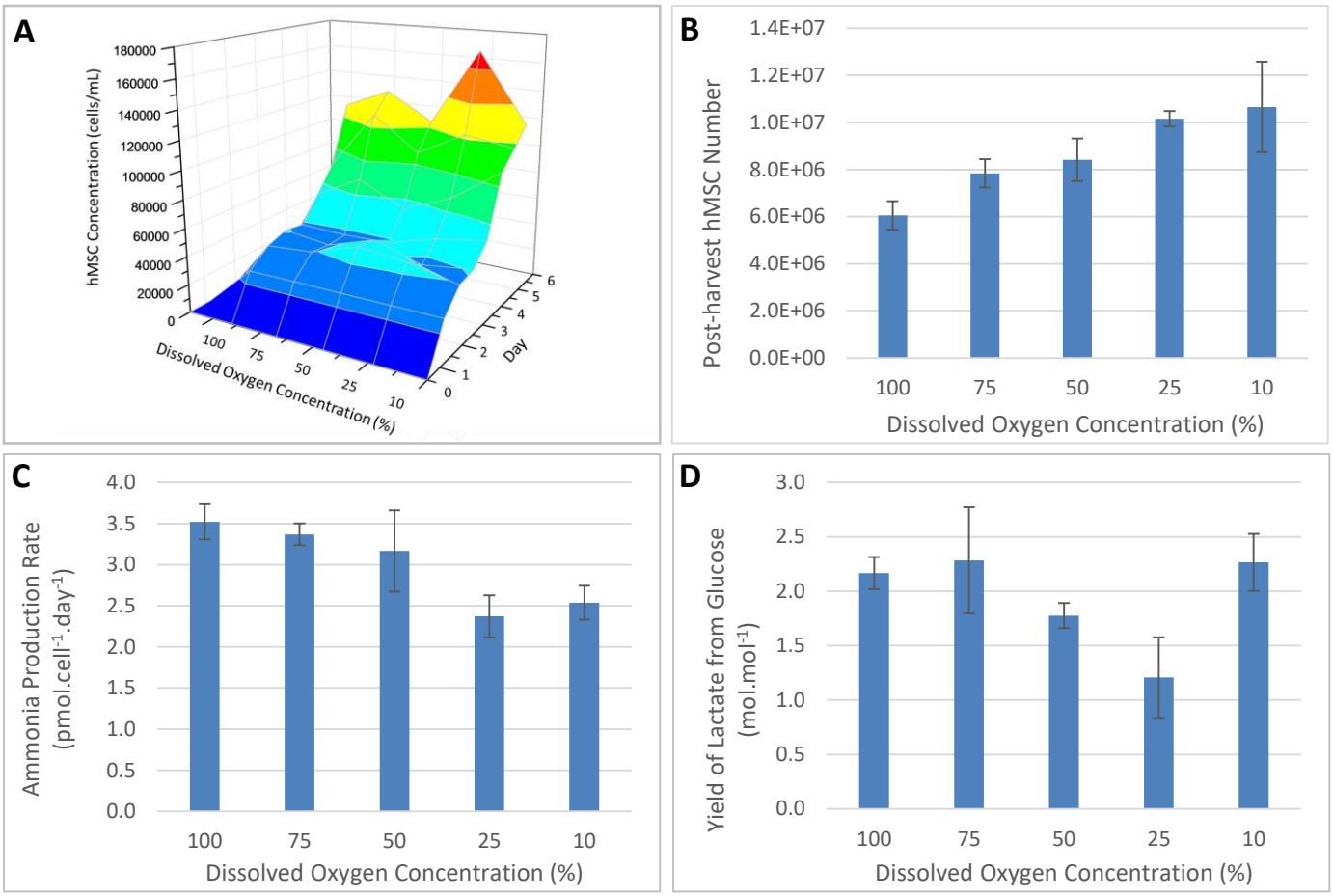


Figure 2 – Effect of dissolved oxygen tension on BM-hMSC growth over six days of culture in the DASbox controlled bioreactor, showing (A) the increased cell number over six days, (B) increased post-harvest cell number at reduced dissolved oxygen concentrations, (C) per cell flux of ammonia and (D) yield of lactate from glucose. Control set-points are 115rpm impeller speed and pH 7.4 with headspace aeration. Data shows mean ± SD, n = 3.

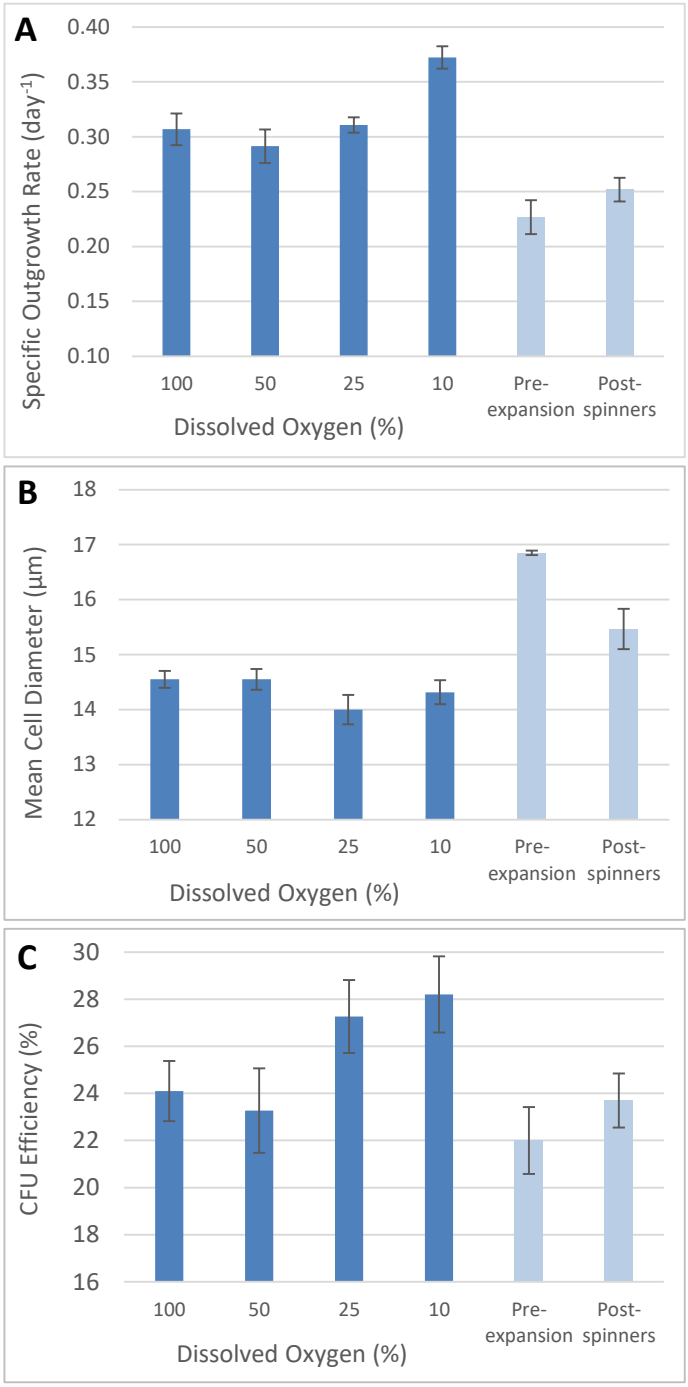


Figure 3 – Post-harvest characteristics of BM-hMSCs from controlled microcarrier culture. Showing (A) increased outgrowth rate (B) reduced mean cell diameter and (C) increased CFU efficiency at reduced DO concentrations. Data shows mean ± SD, n = 3.

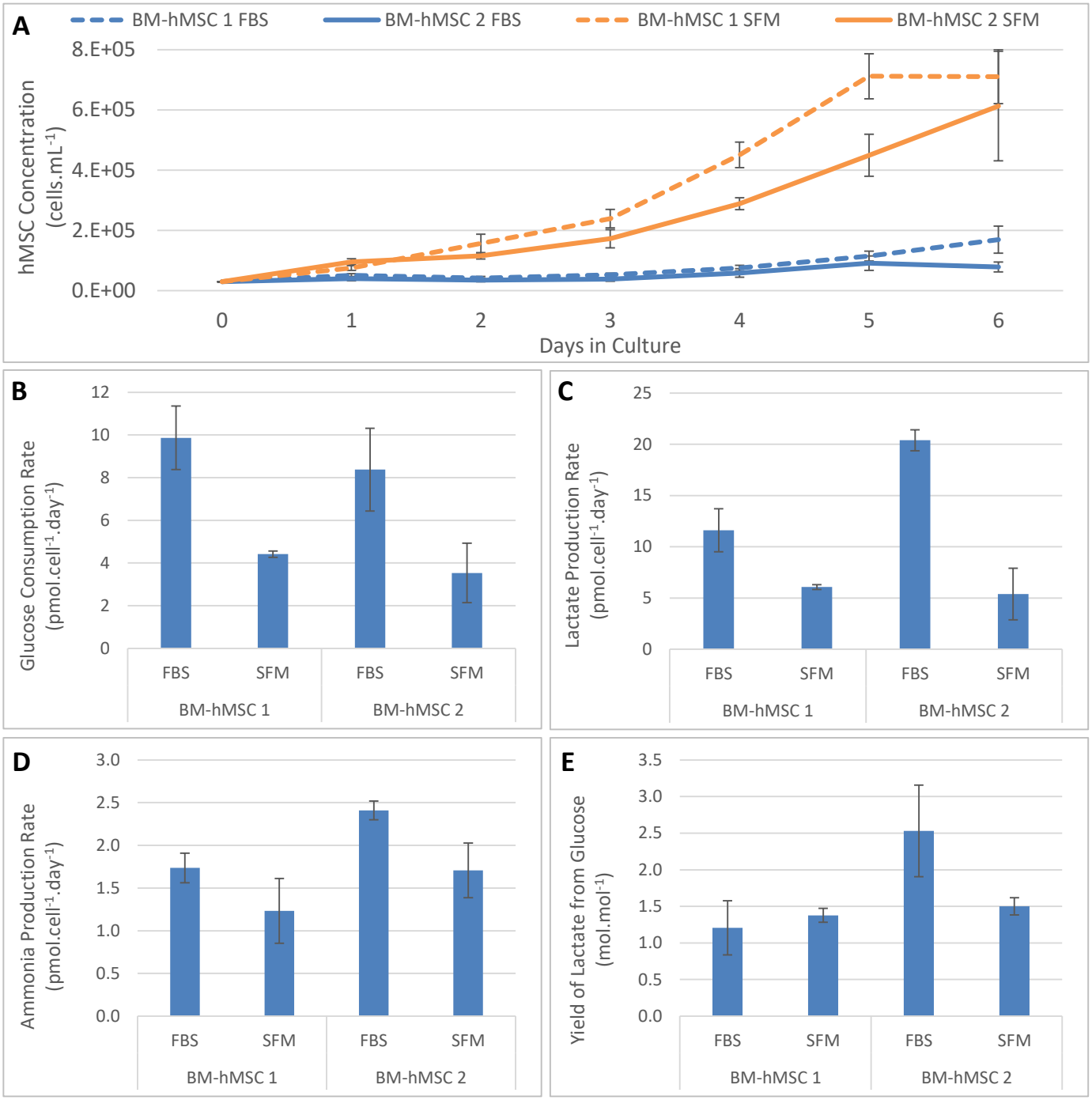


Figure 4 – Comparison of FBS and SFM across two BM-hMSC donors at 115rpm, 25% dissolved oxygen (headspace aeration) and a pH of 7.4 in controlled microcarrier culture. Showing (A) increased growth rate for both donors in SFM, (B) reduced glucose consumption in SFM, (C) reduced lactate production in SFM, (D) reduced ammonia production in SFM and (E) yield of lactate from glucose. Data shows mean ± SD, n = 3.

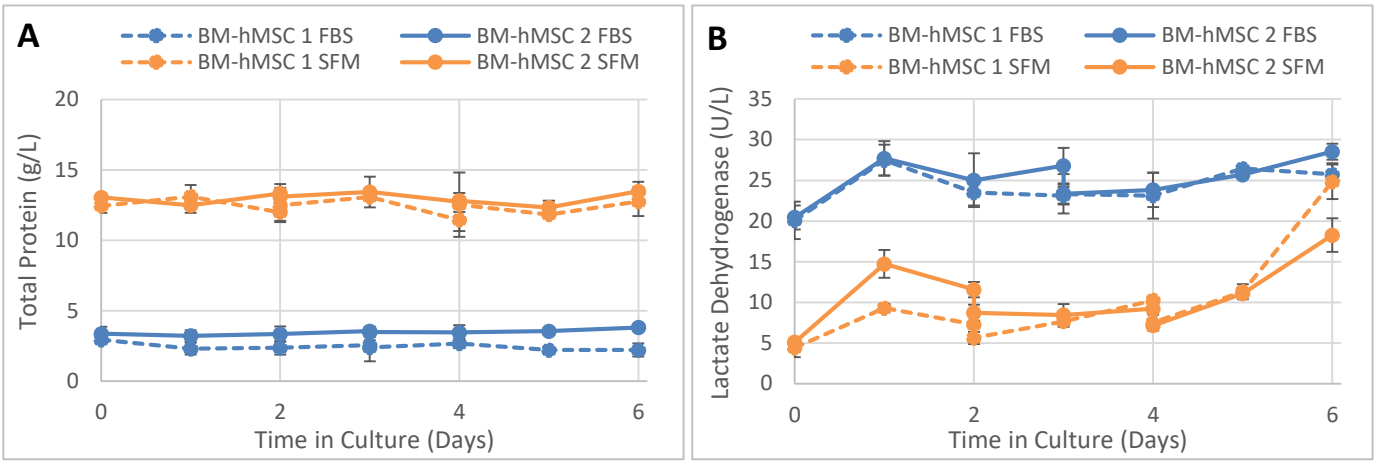


Figure 5 – Comparison of FBS and SFM across two BM-hMSC donors at 115rpm, 25% dissolved oxygen (headspace aeration) and a pH of 7.4 in controlled microcarrier culture. Showing (A) no increase in total protein concentration over six days and (B) increased LDH concentration at the end of SFM culture. Data shows mean ± SD, n = 3.

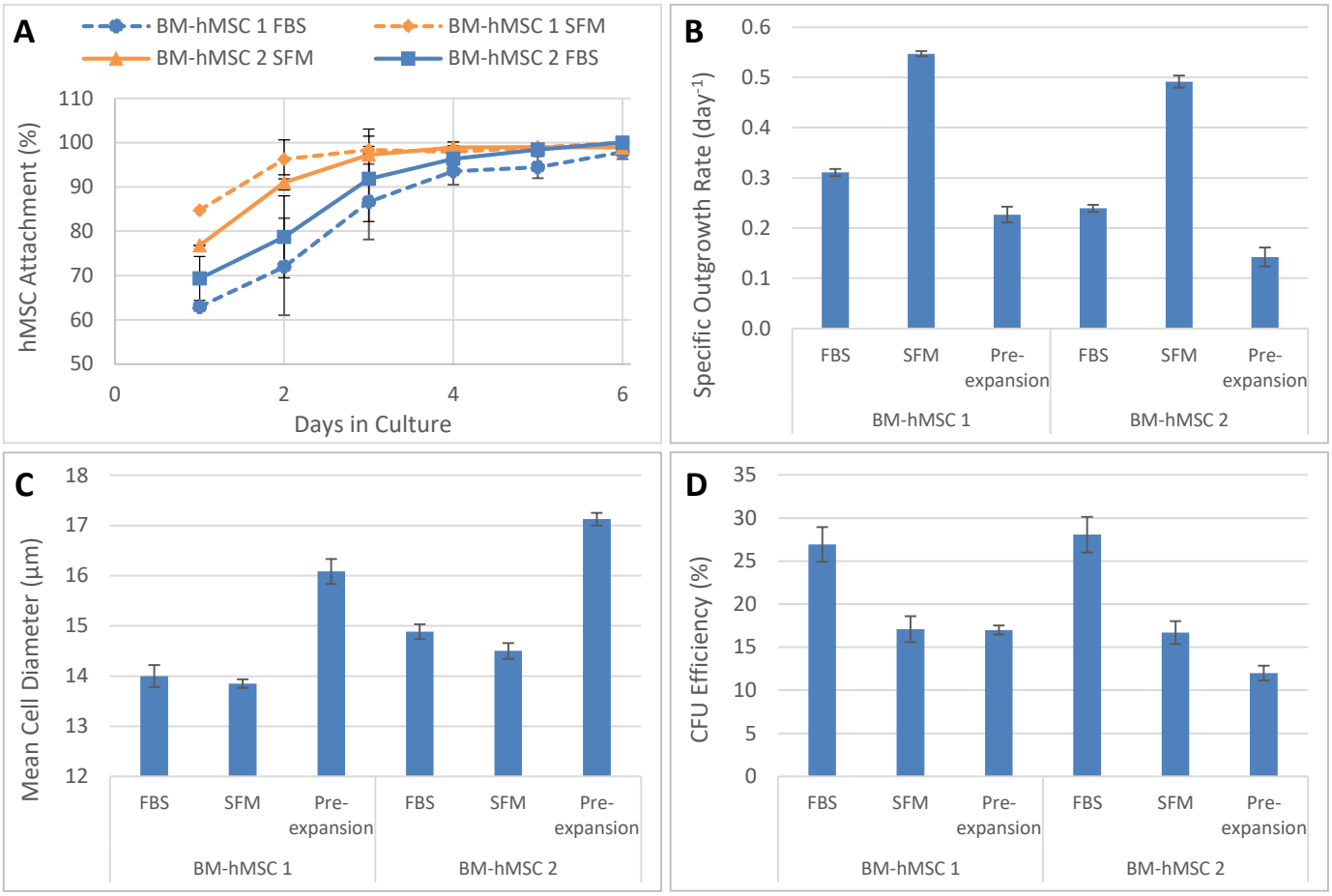


Figure 6 – Attachment rate and post-harvest characteristics of two BM-hMSC donors at 115rpm, 25% dissolved oxygen (headspace aeration) and a pH of 7.4 in FBS and SFM-based controlled microcarrier culture. Showing (A) increased cell attachment to microcarriers in SFM, (B) increased outgrowth rate in SFM, (C) reduced mean cell diameter and (D) maintained CFU efficiency post-harvest. Data shows mean ± SD, n = 3.

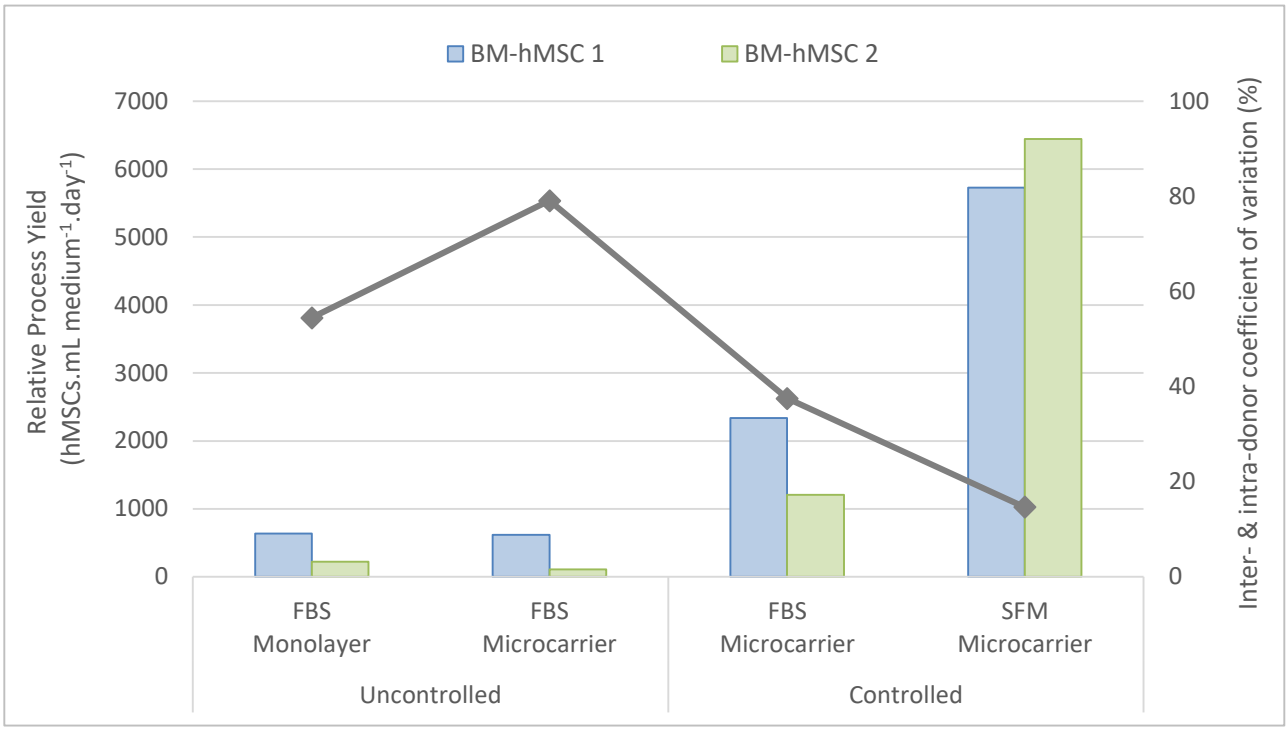
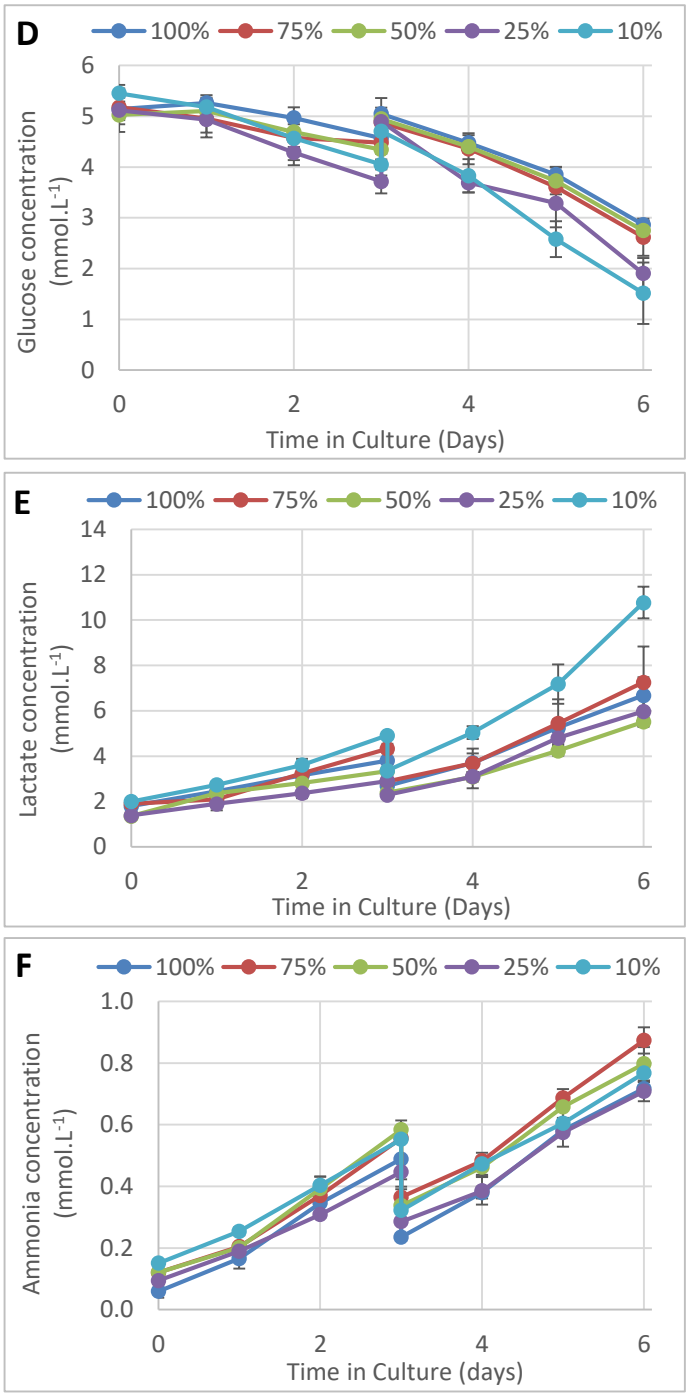
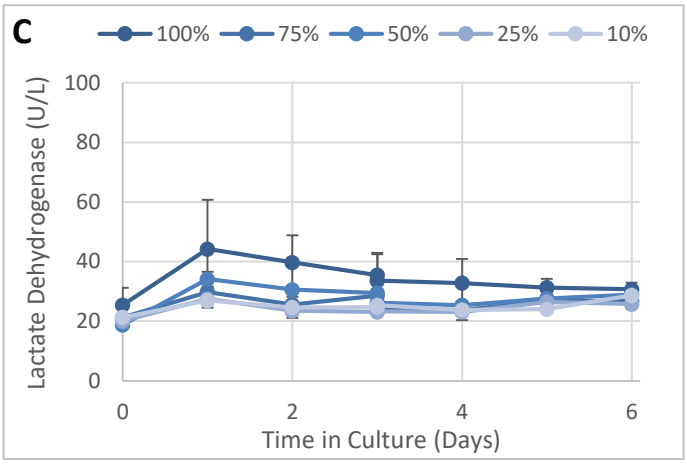


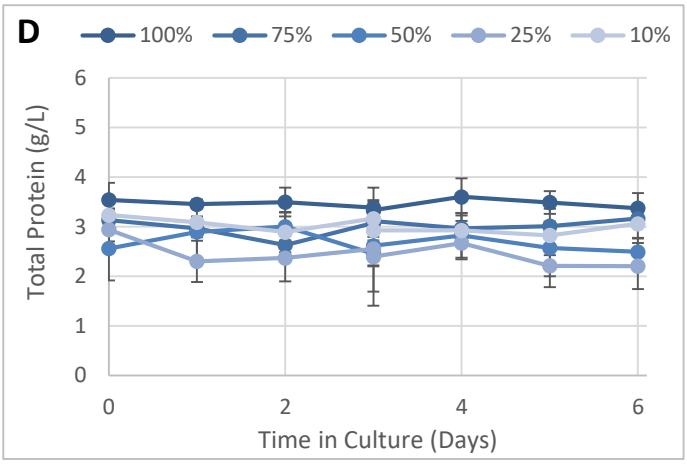
Figure 7 – Impact of a process control system on the process yield from a microcarrier expansion process. Showing that controlled bioreactor processes under serum-free conditions provide much higher yield and consistency between donors. Bars denote process yield in terms of number of cells produced per volume of medium per unit time and the line chart denotes the coefficient of variation between and within donor batch runs.

Supplementary Figure

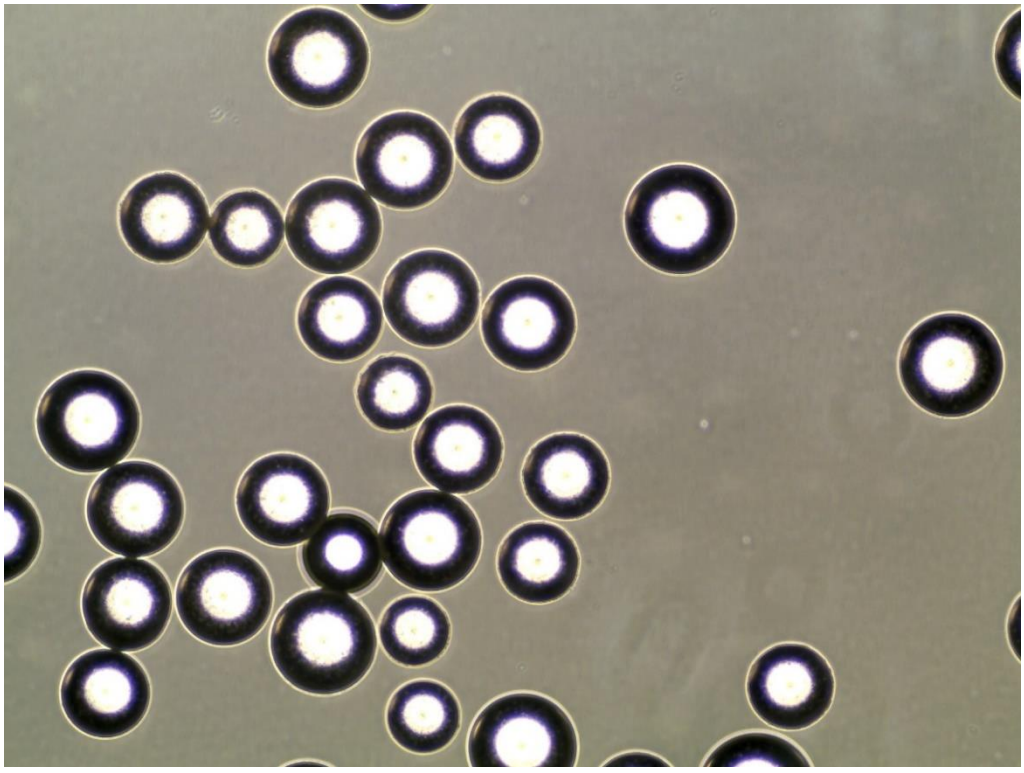


Supplementary figure 1 – Effect of DO concentration and impeller speed on the live metabolite concentrations during BM-hMSC expansion in controlled microcarrier culture. Showing (A) glucose, (B) lactate and (C) ammonia concentrations at various impeller speeds. Also showing (D) glucose, (E) lactate and (F) ammonia concentrations at various DO concentrations. Data shows mean ± SD, n = 3.

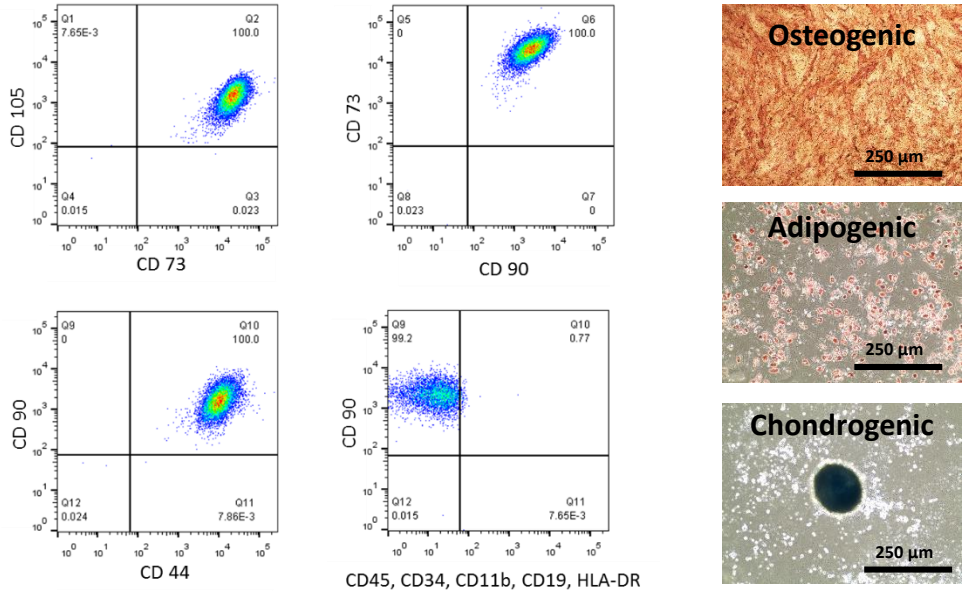




Supplementary figure 2 – Effect of DO concentration, impeller speed and on the metabolite flux of BM-hMSCs in controlled microcarrier culture. Showing no significant increase in (A) LDH concentration or (B) total protein at various DO concentrations and (C) LDH concentration or (D) total protein at various impeller speeds, demonstrating low levels of cell damage during culture. Data shows mean ± SD, n = 3.



Supplementary figure 3 - Phase contrast image of microcarriers post-harvest from the SFM process. Demonstrating that the BM-hMSCs have been successfully removed from the microcarriers during the harvesting process.



Impeller speed (rpm)	% co-expression of immunophenotye markers
80	96.50 ± 0.14
150	96.80 ± 0.28
225	95.80 ± 0.14

Supplementary figure 4 - Verification of post-harvest immunophenotype and differential potential of BM-hMSCs used during this study in accordance with the ISCT minimum criteria.