A Xeno-Free Fed-Batch Microcarrier Suspension Bioreactor System for the Scalable and Economic Expansion of hBM-MSCs

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ABSTRACT

Human Mesenchymal Stem/ Stromal Cells (hMSCs) are considered the “microprocessors” of tomorrow’s Regenerative Medicine (RM) products. Scalable, consistent, and economic manufacturing of hMSCs is required to drive innovation and new technology developments in the RM industry. We have taken the Quality by Design (QbD) approach to develop a scalable, Xeno-Free (XF) bioreactor process to manufacture high quality and functional hMSCs at a consistent final cell population doubling level (PDL 16-20). Our deliberate suspension bioprocess was designed using an optimized and completely XF system comprised of high volume cell banks, bioprocess media, and microcarriers.

In this study, hBM-MSCs were expanded in 0.1L and 3L 3D microcarrier-based bioreactors from PBS Biotech, comparing a partial media exchange to a concentrated XF bioreactor feed (i.e. fed-batch) process. The optimized fed-batch culture process and the half media exchange process yielded comparable cell densities, 3.48±0.38 x10^5 cells/mL and 3.05±0.16 x10^5 cells/mL, respectively. However, the efficiency of the fed-batch system greatly exceeded that of the half media exchange system, yielding 348.3 M cells/L compared to 203.3 M cells/L. A scale up to a 3L fed-batch bioreactor process yielded comparable results to the 0.1L scale, 7.97x10^5 cells/mL and 7.25x10^5 cells/mL, respectively. Critical hMSC quality attributes were also preserved upon the adaptation of cell culture from a 2D to 3D platform. This data demonstrates the development of a cost-effective, scalable, XF fed-batch bioreactor process that can be readily implemented by translational researchers and product developers in the RM industry to generate large quantities of high quality hMSCs

STREAMLINED, SIMPLIFIED, & SCALABLE XF hMSC EXPANSION

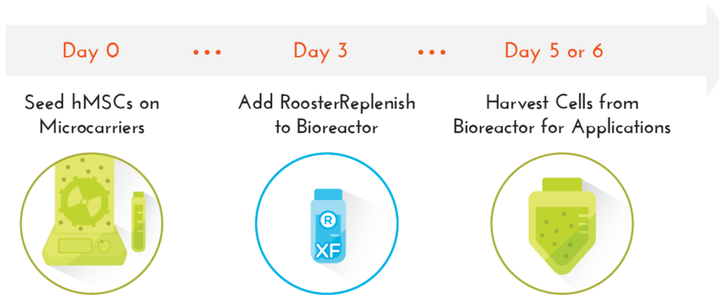

Fig 1: hMSCs seeded into microcarrier bioreactor on day 0 are fed with RoosterReplenish™-MSC on day 3, and are ready for harvest on day 5 or 6 of culture.

FED-BATCH PROCESS OUTPERFORMS ½ MEDIA EXCHANGE & BATCH PROCESSES IN 0.1L PBS BIOREACTORS

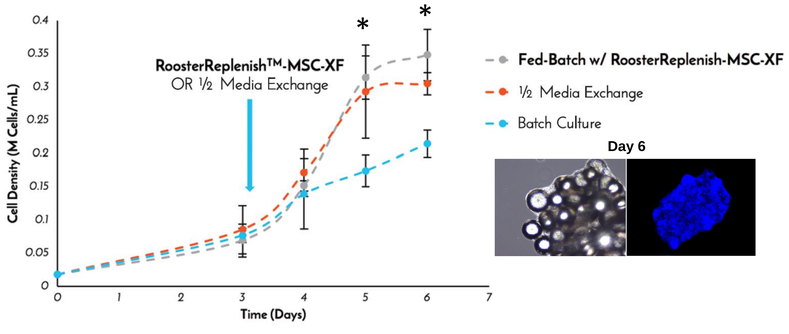

Fig 2: Comparison study of a XF bioreactor process utilizing batch, ½ media exchange, or fed-batch shows a distinct advantage of the fed-batch process on final cell yield and total culture time. ‘*’indicates statistical significance between Fed-Batch and Batch systems at D5 and D6 (α<0.05). Image of hMSC/microcarrier aggregate on Day 6 (left), stained with NucBlue for nuclei (right).

COMPARABLE METABOLITE LEVELS IN ½ MEDIA EXCHANGE AND FED-BATCH BIOREACTOR PROCESSES

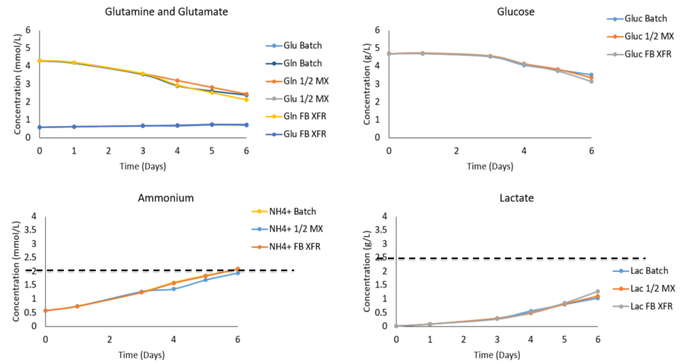

Fig. 3: Concentration of glucose and glutamine maintained above desired levels throughout the culture to support cell expansion. Comparable levels of lactate and ammonia waste accumulation in a fed-batch and media exchange demonstrate the feasibility of fed-batch process in replacement of media exchange process.

FED-BATCH XENO-FREE BIOREACTOR PROCESS MAINTAINS hMSC POTENCY

hMSCs in Bioreactors Maintain Critical Quality Attributes

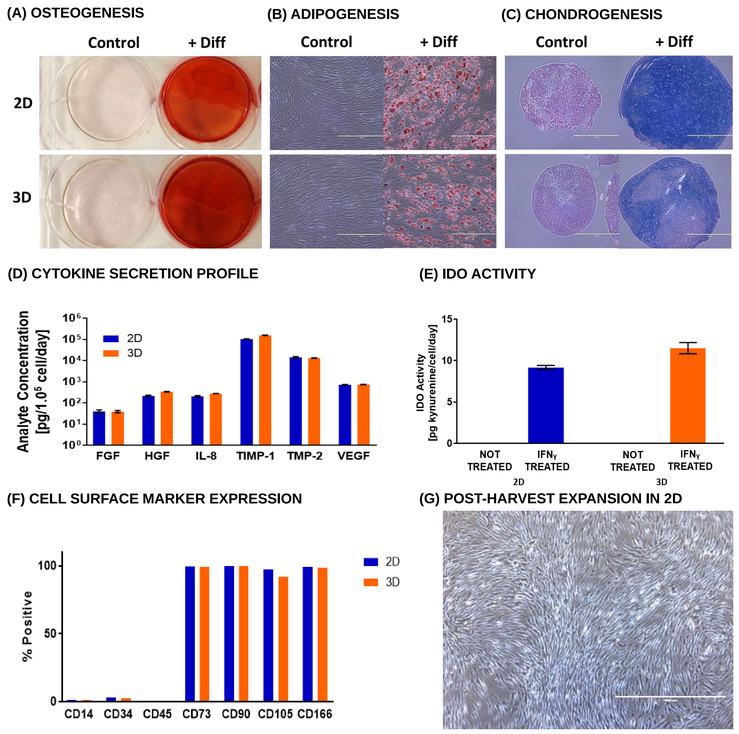

Fig 4: XF hMSCs expanded in the fed-batch bioreactor maintained their tri-lineage differentiation potential to (A) osteo-, (B) adipo-, and (C) chondrocytes, similar to cells expanded in 2D culture. XF hMSCs maintained their (D) cytokine secretion profile, (E) inducible indoleamine 2,3-dioxygenase (IDO) activity when stimulated with interferon-gamma (IFNγ), (F) cell surface marker expression identity, and (G) typical hMSC morphology post bioreactor harvest.

COMPARABLE CELL EXPANSION PERFORMANCE WHEN SCALED TO A 3L BIOREACTOR

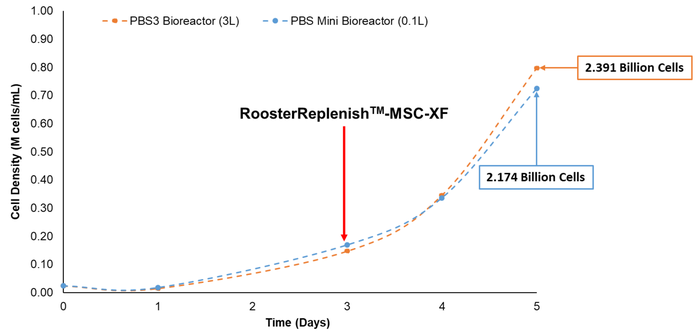

Fig 5: The 0.1L PBS bioreactor culture process was scaled to the 3L development system, with controlled pH, PO2, temperature, and gas. Results demonstrate the scalability of the bioreactor process performance to meet potential clinical and commercial demand.

RAPID EXPANSION OF hMSCs ON MICROCARRIERS IN BIOREACTORS

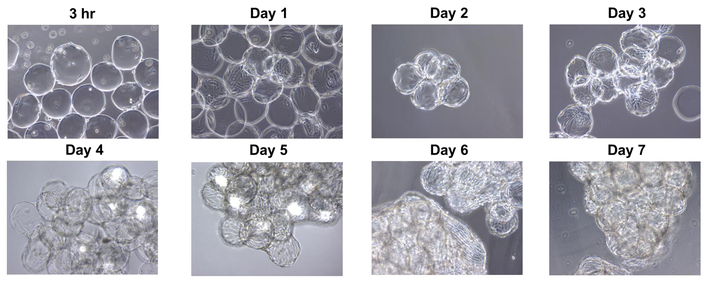

Fig 6: hMSCs expanding in bioreactor culture to form cell/microcarrier aggregates.

ENHANCED MEDIA PRODUCTIVITY USING A FED-BATCH BIOREACTOR SYSTEM

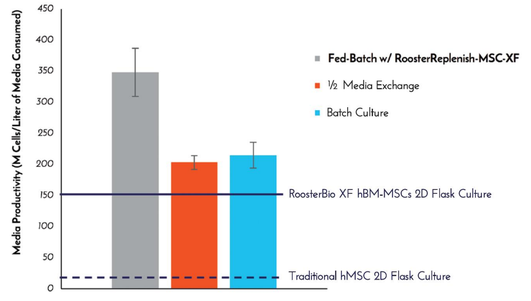

Fig 7: Fed-batch culture using RoosterReplenish-MSC-XF is the most efficient and productive culture feeding regimen, outperforming either ½ media exchange or batch culture by over 70%, and achieving up to 350 M cells/L in media productivity. This productivity translates to a significant cost savings for bioreactor-based hMSC manufacturing.

CONCLUSIONS

- This work demonstrates an efficient, scalable XF bioreactor culture system to manufacture XF hMSCs
- A fed-batch bioreactor process enhances media productivity, is more cost-effective, and less labor-intensive for large scale expansion of hMSCs in suspension culture.
- hMSC critical quality attributes were maintained in the 3D PBS bioreactor system

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Work Funded by the Medical Technology Enterprise Consortium