A Fed-Batch Microcarrier Suspension Bioreactor System for the Scalable Expansion of hBM-MSCs

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ABSTRACT

Human bone marrow-derived Mesenchymal Stem Cells (hBM-MSCs) have been recognized as potential patient-specific drugstores, and will be a key raw material for future therapeutics, engineered tissues, and medical devices. Production technologies such as suspension bioreactors are robust, scalable platforms for generating hundreds of billions of cells per manufacturing run to meet the demand for these applications. We have taken the Quality by Design (QbD) approach to design scalable hMSC bioreactor processes that consistently maintain the final cell population doubling level (PDL) within the recommended level of 16-20 range, even as we scale to hundreds of liters of culture. We investigated the use of a suspension bioreactor, along with the use of a concentrated bioreactor feed to replenish nutrients and growth factors depleted from growth medium, for the scalable expansion of hBM-MSCs. The use of a high-performance growth media and a concentrated feed in a fed-batch system not only maximizes culture yield, but it minimizes the time required for media preparation, media exchange, and contamination risk associated with process manipulation. hBM-MSCs grown on microcarriers in bioreactors yielded >3x10^5 cells/ml within 6 days of culture, with either half media exchange or a fed-batch process. The metabolite levels of lactate and ammonia were maintained below growth-suppression concentration of 2g/L and 2.5mM, respectively, with both feed regimens. In addition, with the adaptation of cell culture from a 2D to 3D platform, we confirmed the maintenance of critical hMSC functional properties including angiogenic cytokine (FGF, HGF, IL-8, TIMP-1, TIMP-2, and VEGF) secretion, tri-lineage differentiation, and immunomodulatory potential. Thus, microcarrier suspension culture of hMSCs, with a bioreactor feed in lieu of full or partial media exchanges, will scale hMSC culture, while streamlining the process, to provide significant time and cost savings for translational researchers in Regenerative Medicine and Tissue Engineering.

STREAMLINED & SIMPLIFIED UPSCALED HMSC EXPANSION

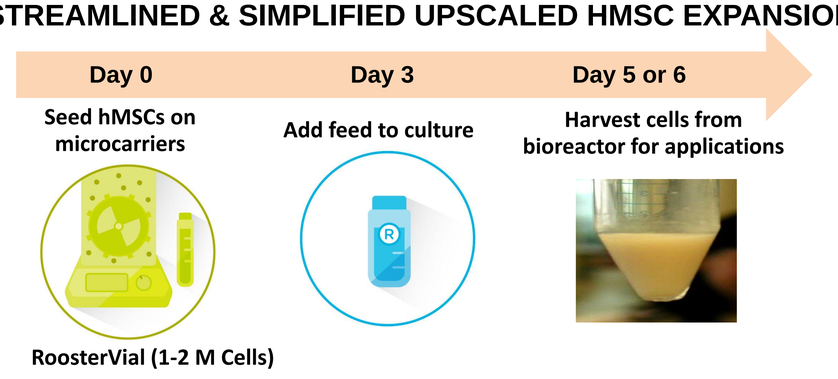


Fig 1: hMSCs seeded into microcarrier bioreactor on day 0 are fed with RoosterReplenish-MSC on day 3, and are ready for harvest on day 5 or 6 of culture.

hMSC EXPANSION ON MICROCARRIERS

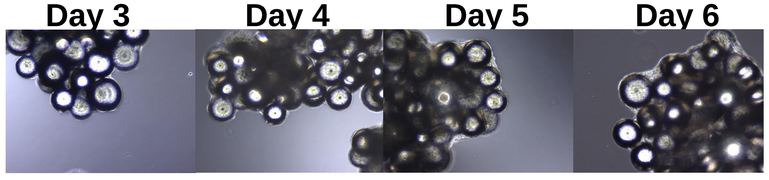


Fig 2: hMSCs culture were sampled and monitored for cell growth and agglomeration on microcarriers. hMSC proliferation were observed by the formation of cell-bead agglomerates during expansion.

FED-BATCH PROCESS OUTPERFORMS ½ MEDIA EXCHANGE AND BATCH PROCESSES IN BIOREACTORS

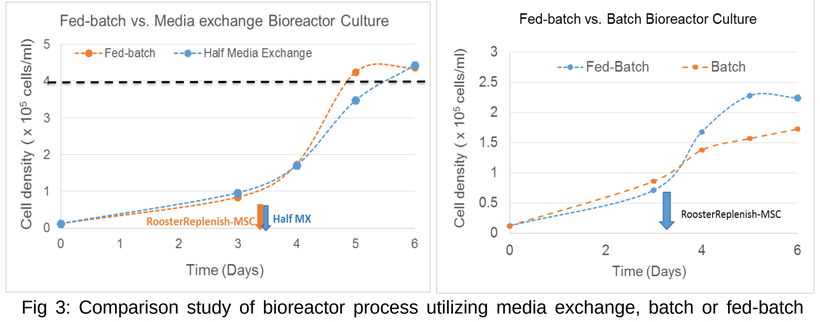


Fig 3: Comparison study of bioreactor process utilizing media exchange, batch or fed-batch show a distinct advantage of fed-batch process on final cell yield and total culture time to confluency when tested with cells from different donors.

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

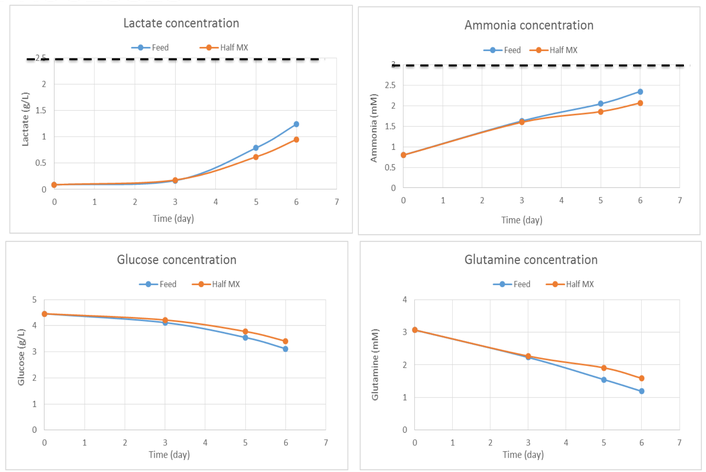
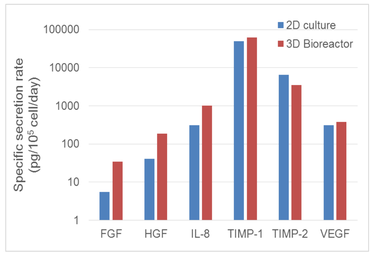


Fig. 4: Concentration of glucose and glutamine maintained at desired levels throughout the culture to support cell expansion. Comparable levels of lactate and ammonia waste accumulation in a fed-batch and media exchange demonstrate the feasibility of fed-batch process for media feed regime in replacement of media exchange process.

hMSCs IN BIOREACTOR CULTURE MAINTAINED THEIR POTENCY AND FUNCTIONALITY

I. Cytokine Secretion



II. IDO Activity

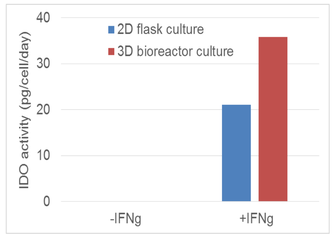


Fig. 5: (I) Comparable level of cytokine secretion from hMSCs cultured in flask and fed-batch bioreactor demonstrate cells maintain functionality and (II) hMSCs maintained their inducible indoleamine 2,3-dioxygenase (IDO) activity when treated with interferon-gamma (IFNg).

III. Tri-lineage Differentiation

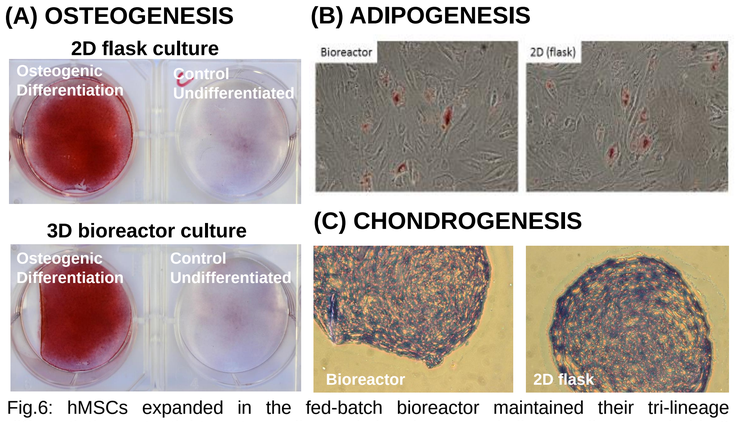


Fig.6: hMSCs expanded in the fed-batch bioreactor maintained their tri-lineage differentiation potential to osteo- adipo and chondrocytes, similar to cells expanded in 2 dimensional culture.

TRANSLATION TO A XENO-FREE BIOREACTOR SYSTEM—CELLS, MEDIA, MICROCARRIERS

I. Fed-Batch Process Outperforms ½ Media Exchange and Batch Processes in Bioreactors

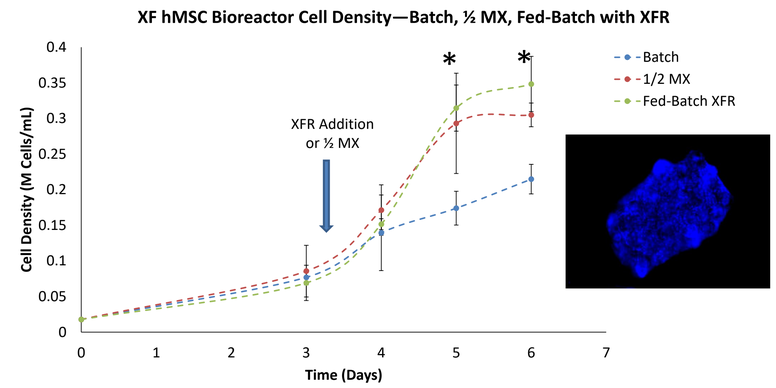


Fig 7: Comparison study of a Xeno-Free bioreactor process utilizing batch, ½ media exchange, or fed-batch shows a distinct advantage of the fed-batch process on final cell yield and total culture time. '*'indicates statistical significance between Fed-Batch and Batch systems at D5 and D6 (α<0.05). Image of hMSC/microcarrier aggregate on Day 6, stained with NucBlue for nuclei.

II. XF hMSCs in Bioreactor culture maintained functionality

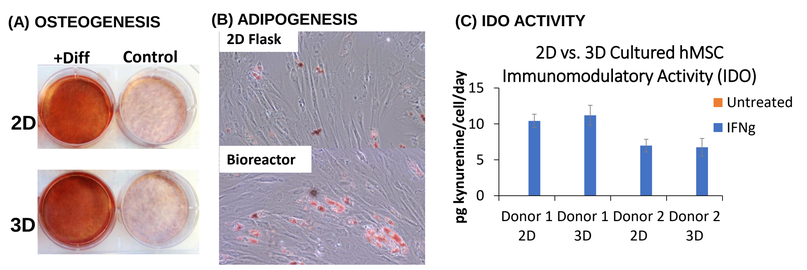


Fig 8: Xeno-free hMSCs expanded in the fed-batch bioreactor maintained their tri-lineage differentiation potential to (a) osteo- and (b) adipocytes, similar to cells expanded in 2D culture. (c) Xeno-Free hMSCs maintained their inducible indoleamine 2,3-dioxygenase (IDO) activity when treated with interferon-gamma (IFNg).

ENHANCED MEDIA PRODUCTIVITY IN A FED-BATCH SYSTEM COMPARED TO BATCH OR ½ MEDIA EXCHANGE BIOREACTOR SYSTEMS

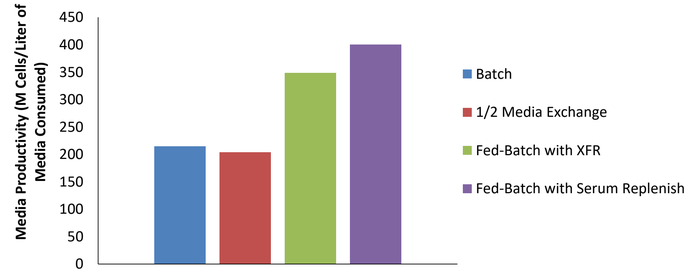


Fig 9: A 1.5 to 2-fold increase in media productivity was observed for a fed-batch process (xeno-free and serum) compared to half media exchange or batch culture system. This translates to a significant cost saving in a bioreactor culture where media is the cost driver of the process.

CONCLUSIONS

- hMSC expansion in bioreactors yielded 350-400,000 cells/Ml, demonstrating scalability and better product economics in both systems
- Both serum and Xeno-Free hMSCs maintained their quality attributes when expanded in scalable fed-batch microcarrier bioreactor systems, comparable to 2D cultures
- 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.