A Scalable Xeno-Free Microcarrier-based Suspension Bioreactor System for Biomanufacturing of Human Mesenchymal Stem Cells (hMSCs) for Regenerative Medicine

Josephine Lembong, Robert Kirian, David Wang, Jon A. Rowley, and Taby Ahsan
RoosterBio, Inc., 5295 Westview Drive #275, Frederick, MD 21703

Statement of Purpose: An economical biomanufacturing paradigm for human mesenchymal stem/stromal cells (hMSCs) is in critical need, as indicated by over 800 clinical trials investigating the use of hMSCs for regenerative medicine. To meet the growing demand for clinical manufacturing, a scalable process and production platform that can generate billions to trillions of cells per manufacturing lot is needed. Suspension bioreactors show great promise in reaching commercially-viable working volumes, however, scalability of cell production remains an issue. Overcoming this challenge is necessary to drive widespread adoption of this culture system for hMSCs. We have taken a Quality by Design (QbD) approach to develop a scalable xeno-free (XF) hMSC bioreactor process that maintains the final cell population doubling level (PDL) within the recommended range of 16-20 to ensure product quality. Our strategic bioprocess was designed using high volume XF cell banks and XF microcarriers, using an optimized fed-batch XF media system (bioprocess media and bioreactor feed), and combined in a scalable bioreactor system for streamlined production at different culture scales.

Methods: Human Bone Marrow-Derived MSCs (hBM-MSC) were cultured on Corning® Synthemax® II microcarriers (Corning, Corning, NY) in suspension at different scales using vertical wheel bioreactors (PBS Biotech, Camarillo, CA). In both the PBS Mini Bioreactor (0.1L-BR) and PBS-3 Bioreactor (3L-BR), with working volumes of 0.1L and 3L, respectively, cell inoculation was performed using 1/3 of the working volume and cell attachment was facilitated through intermittent agitation cycles. The 0.1L-BR was laid on an agitation-controlled base in a standard 5% CO_2 incubator, while the 3L-BR was directly connected to gases (air, CO_2, O_2, N_2) and equipped with temperature, pH, and DO control systems. Fresh culture media was added to the full working volume to reach the initial cell density of 23,333 cells/ml and then steady agitation was initiated. On Day 3, a bioreactor feed was added at 2% of the working volume. Daily sampling for cell counts and media analysis was performed to monitor cell growth, nutrition, and waste profiles.

Results: We have developed a XF process for hMSC culture in suspension, in which hMSCs are seeded onto microcarriers in a bioreactor on Day 0, fed on Day 3, and harvested on Day 5, resulting in a cell yield of >0.5M cells/ml. Process optimization in the small scale (0.1L) resulted in cell-microcarrier attachment efficiency of >60%. We demonstrated that this process was directly scalable to development volumes (3L), maintaining a similar cell yield within the 5 days of culture with no media exchange. Comparable nutrient and waste metabolite levels, pH, and cell growth curves were also observed at both scales, indicating that similar strategies can be used across bioreactor systems to control nutrients and waste in the media. In addition, cells harvested from all bioreactor scales were comparable to 2D controls of similar PDL, maintaining hMSC critical quality attributes of osteogenic, adipogenic, and chondrogenic differentiation potential, as well as functional attributes of angiogenic cytokine (FGF, HGF, IL-8, TIMP-1, TIMP-2, and VEGF) secretion and inducible immunomodulatory potential (as measured by functional IDO activity). Our process, along with the environmental cues provided by the microcarriers and bioreactor system, supports the expansion of XF hMSCs in a scalable bioreactor culture platform. Thus, significant time and cost savings can be realized for translational researchers and product developers in the regenerative medicine, tissue engineering, and cell therapy fields.

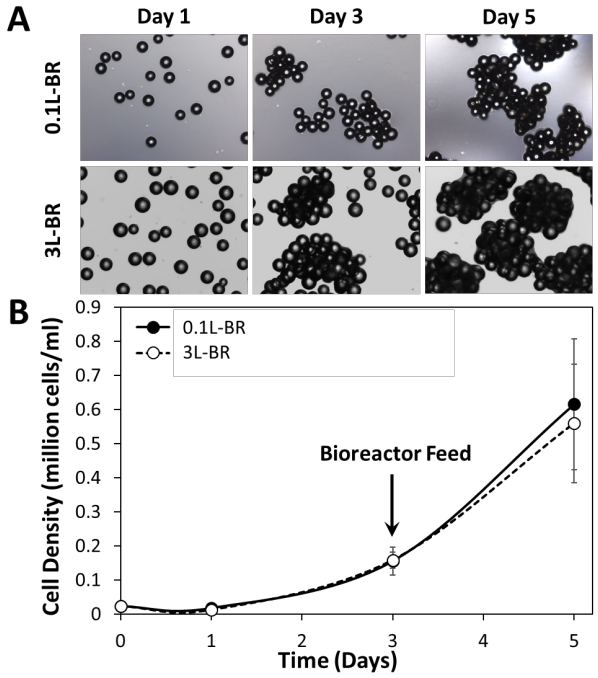

Figure 1. (A) hMSC cultures were sampled and monitored for cell growth on microcarriers on Day 1, 3, & 5 of bioreactor culture. (B). Growth profiles of XF hBM-MSCs are comparable across scales of microcarrier-based suspension culture, achieving cell yields of >0.5M cells/ml within 5 days.

Conclusions: We have previously shown that a fed-batch bioreactor process enhances media productivity, and is more cost-effective, and less labor-intensive for large scale expansion of hMSCs in suspension culture. Here we showed that our fed-batch XF bioreactor process achieves hMSC yields of up to 0.5M cells/ml within 5 days at both the small (0.1L) and development (3L) scales while maintaining typical phenotypic and functional properties of hMSCs. We intend to continue to scale this process up to pilot (15L) and production scale (80L) bioreactors, which are critical steps to generating the cell numbers necessary for clinical therapies.