SCALE UP OF ALLOGENEIC CELL THERAPY MANUFACTURING IN SINGLE-USE BIOREACTORS: CHALLENGES, INSIGHTS AND SOLUTIONS

Brian Lee, President, PBS Biotech, CA, USA
blee@pbsbiotech.com
Yas Hashimura, PBS Biotech, CA, USA
Sunghoon Jung, PBS Biotech, CA, USA
Maximilian Lee, PBS Biotech, CA, USA

Key Words: single-use bioreactors; cell therapy; scale up; mesenchymal stem cells; human induced pluripotent stem cells; Vertical-Wheel bioreactors; microcarriers; aggregates.

Allogeneic cell therapy products have enormous potential to treat a wide range of unmet medical needs, with various drug candidates getting closer to commercialization. However, the limited manufacturing capacity of 2D planar technology to meet commercial demands could be a potential bottleneck for the future success of the emerging cell therapy industry. With the benefits of high volumetric productivity, operational controllability, and scalability of cell culture processes for therapeutic protein manufacturing, single-use bioreactors are recognized as promising solutions for large-scale manufacturing of cell therapy products. However, cell therapy products have unique bioprocessing requirements that are different from protein manufacturing. Not only is the final product the cell itself, but many cell therapy products are anchorage-dependent and grow on microcarriers or as cell aggregates. These microcarriers and cell aggregates are much larger than free-floating single cells and require comparatively greater power input to be suspended in a bioreactor, which can lead to hydrodynamic sheer stress and damage to cells. While various cell types require different bioreactor processes, a single-use bioreactor with the following capabilities would be greatly beneficial for reliable scale up of cell therapy manufacturing. One capability is to homogeneously and efficiently suspend large particles in a low shear environment. Another is to provide evenly distributed dissipation energy inside the vessel, leading to uniform aggregate size formation. Finally, both of these capabilities should be reproducible at larger volumes so that micro-environments inside the bioreactors are consistent across various working volumes.

A novel, single-use Vertical-Wheel bioreactor system was designed and introduced in an attempt to provide these capabilities. Experiments with various cell types such as human mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cells (PSCs), and chondrocytes have been performed in different sizes of Vertical-Wheel bioreactors with the following results. Comparable cell growth of MSCs (550,000 to 600,000 cells/mL on day 5) in a xeno-free microcarrier culture was achieved in three different scales of bioreactors (0.1L, 3L, and 15L). After the cell expansion phase, in-vessel cell dissociation from microcarriers using Trypsin was performed in the 15L Vertical-Wheel bioreactors with a greater than 90% harvest yield. Aggregates of ESCs grown in 0.5L Vertical-Wheel bioreactors were shown to be more uniform in size compared to aggregates grown in stirred-type spinner flasks. Furthermore, the narrow range of aggregate sizes (150-250 microns) after three days of cultivation in 0.5L Vertical-Wheel bioreactors was reproduced at larger 3L scale. The size of PSC aggregates could also be controlled by adjusting agitation rate, with higher speeds resulting in smaller aggregates and lower speeds in larger aggregates. In addition, pluripotency of PSC aggregates was maintained after cell expansion, as indicated by specific surface marker identifiers. Directed differentiation of PSCs in a single-use bioreactor is another important challenge of manufacturing scale up. Vertical-Wheel bioreactors have been used to successfully differentiate PSCs into different types of target cells, such as insulin-producing SC-islets or cerebellar organoids.

In order for single-use bioreactors to become the standard manufacturing platform for cell therapy products, an ideal bioreactor system should be able to deliver highly productive, reliable, and reproducible cell culture performance at commercial scale. Further details regarding manufacturing challenges and experimental data will be discussed in this presentation.