Musculoskeletal injuries are the leading cause of lameness and loss of performance in horses and conventional treatments are often associated with high rates of re-injury. Mesenchymal Stem Cells (MSCs) have shown promise for the treatment of such injuries in horses. Currently, the majority of studies are focused on the use of either bone-marrow derived or adipose-derived MSCs. However, equine cord-blood derived MSCs (eCB-MSCs) also provide a promising alternative, due to their high proliferation potential, ability to differentiate towards the chondrogenic lineage, and comparable immune-modulatory properties.

Static adherent culture of eCB-MSCs has limited potential to produce sufficient cell numbers for large-scale research studies and possible commercial distribution. Expansion of cells in stirred suspension bioreactors using microcarriers as a scaffold has the potential to generate a large number of cells, using a significantly smaller space, under highly controlled conditions, with reduced time, labour, and monetary requirements. A robust protocol is required for the expansion of eCB-MSCs for use in large research studies and commercial applications.

Initially, the hydrodynamic environment in the 10mL and the 100mL bioreactors was modeled using COMSOL Multiphysics software. The volume distributions of shear stress and energy dissipation rate in the bioreactors were calculated and used to determine the operating conditions that would create similar conditions within both scales of bioreactors.

Next, eCB-MSCs were expanded in 10mL stirred suspension bioreactors and run at 60rpm and 80rpm with two different impeller geometries: paddles and rounded edges. The bioreactors were loaded at 4500 cells/cm², and 2g/L microcarriers. The cells at different operating conditions in the 10mL bioreactors achieved varying population doubling times ranging from 0.8d to 1.1d with an average of 0.9d and initial cell attachment ranging from 5000 cells/cm² to 7700 cells/cm². The different speeds and geometries produced varying results with maximum attached cell densities from 35,000 to 50,000 cells/cm² in the bioreactors, compared to maximum cell densities of 44,000 cells/cm² achieved in static growth.

The expansion of eCB-MSCs was then scaled up in 100mL stirred suspension bioreactors with no direct pH or dissolved oxygen control, using 4500 cells/cm² and 2g/L microcarriers, with a speed of 40rpm. At this larger scale, the initial cell attachment was 6900 cells/cm² compared to 6300 cells/cm² for the 10mL bioreactor. With respect to initial cell attachment, the 100mL bioreactor at 40rpm was most similar to the condition of 80rpm with round edge impeller geometry. The highest attached cell density in the 100 mL vessel was 70,000 cells/cm². The 100mL uncontrolled bioreactor at 40rpm achieved the most similar results to the 10mL bioreactor run at 60rpm with paddled geometry, with respect to population doubling time with a doubling time of 0.93d for the 10mL bioreactor compared to 0.92d for the 100mL bioreactor.

Finally, the eCB-MSCs were expanded in 100mL stirred suspension bioreactors at 4500 cells/cm², 2g/L and 40rpm with pH and oxygen controlled at 7.4 and 21% DO, respectively, using the DASGIP bioreactor control system. This series of experiments revealed that eCB-MSCs can be expanded in stirred suspension bioreactors.