Developing integrated single-use upstream and downstream platform processing options for allogeneic cell therapy applications: linking the stirred tank bioreactor to the TFF

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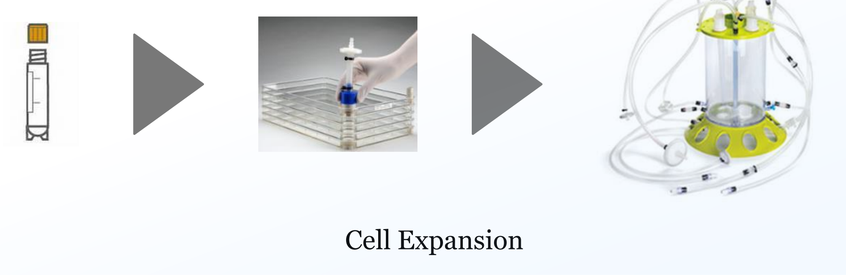
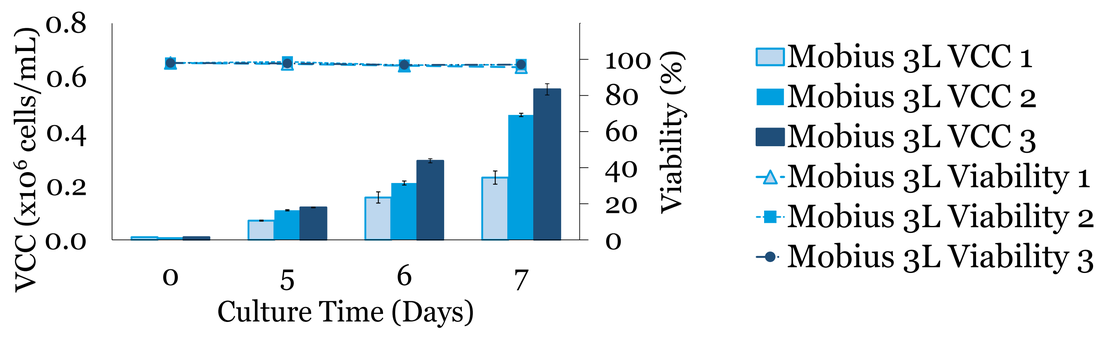
Aim

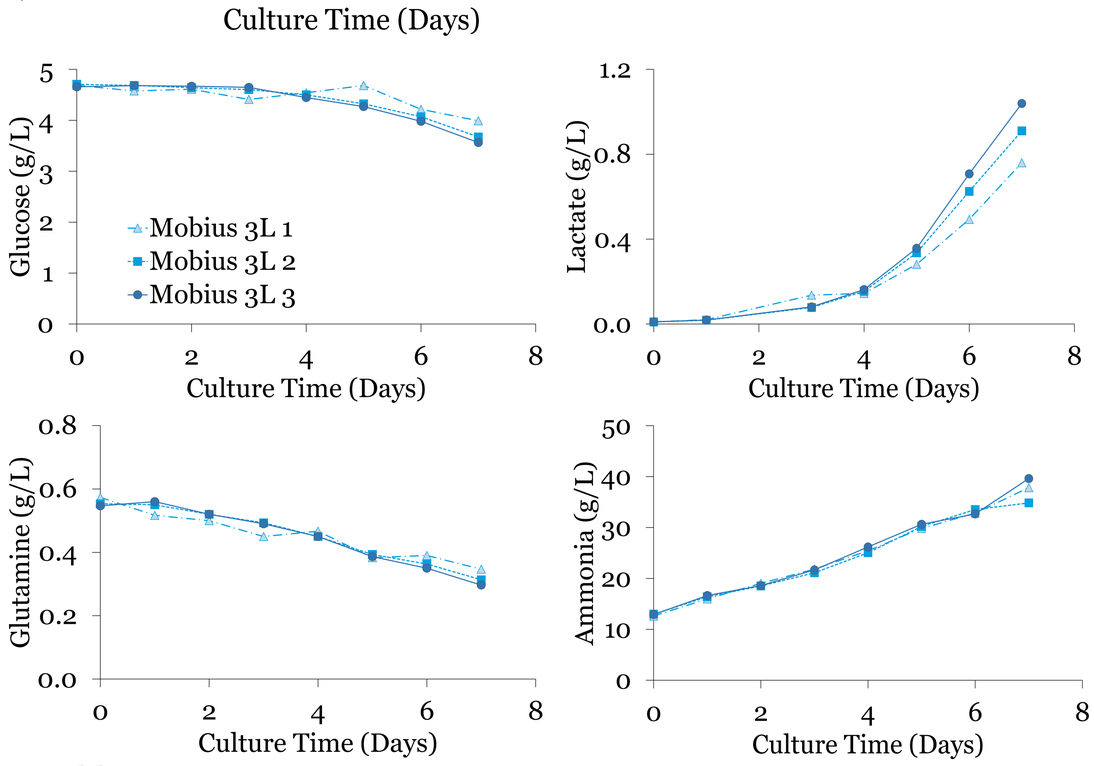
Integration of suitable closed systems for a seamless transition between cell culture, cell product harvest and the following concentration, diafiltration and formulation operations has been a challenge for the development of allogeneic cell therapies. The Cell and Gene Therapy Catapult aims to demonstrate an option for integrating cell culture and harvest operations for microcarrier-based culture of human bone marrow derived Mesenchymal Stem Cells (hMSC). Cell expansion and separation from microcarriers was followed by the screening of suitable tangential flow filtration (TFF) options for cell concentration and washing required prior to product formulation.



Cell Expansion

Bone marrow derived hMSCs were expanded in 2D using a 4 layer cell factory for 4 days, prior to seeding into the Mobius 3L (EMD Millipore) stirred tank bioreactor (STR) on collagen microcarriers (Pall) at 15g/L. RoosterBio medium was used throughout the cell expansion. RoosterReplenish cell culture feed was added at 2% v/v on day 3 of the culture. Viable cell concentration (VCC) was quantified throughout the culture period, where an average cell expansion of 35 fold was achieved over the 7 days.





Cell Harvest

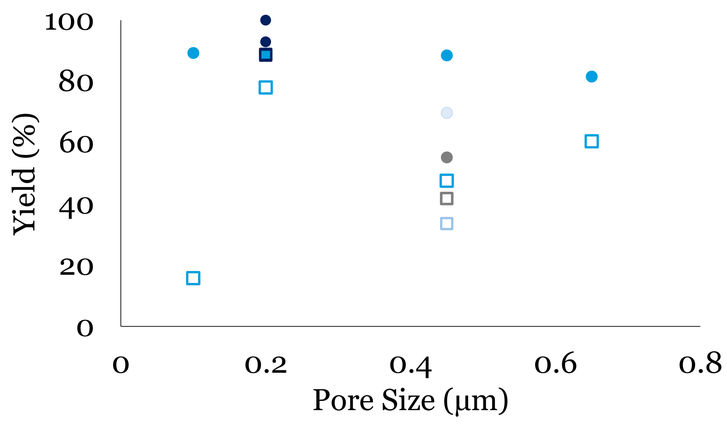
Microcarriers were settled inside the vessel on day 7 of culture. They were washed using 1L of dPBS. TrypLE Express was added to the Mobius 3L vessel, impeller rotation rate of 50RPM and dissolved oxygen control at 37°C was used for 20 minutes. The TrypLE Express was quenched using a 1:1 ratio of spent medium. Triplicate samples of 10mL were removed from the vessel and passed through a 70µm strainer. The remaining cell-microcarrier suspension was passed through a harvest device, connected to the Mobius 3L in a closed manner. The cell harvest device achieved equivalent cell-microcarrier separation to the lab scale strainer.

Harvest Volume	Cell Number	Device Yield Vs Cell Strainer
1.8L	354x10⁶	109%

Cell Concentration

TFF device modules can be incorporated into a closed system TFF design suitable for manufacturing scales of 1-50L.

Key variables which may impact cell product recovery and quality performance include device type (material and design), operating conditions and pore size. AKTA Crossflow was used to screen a range of TFF modules to select the optimum option for concentration and wash of hMSC cells. The screening was carried out at a shear rate of 3000s^{-1} using controlled flux of 30LMH. HEK293 cells at 1x10^6 cells/mL were used to provide model cell material for the screening and showed the 0.2µm GE hollowfibre cartridge to retain cell yield >80%. 10 fold hMSC cell concentration and wash was shown to achieve a 90% yield.

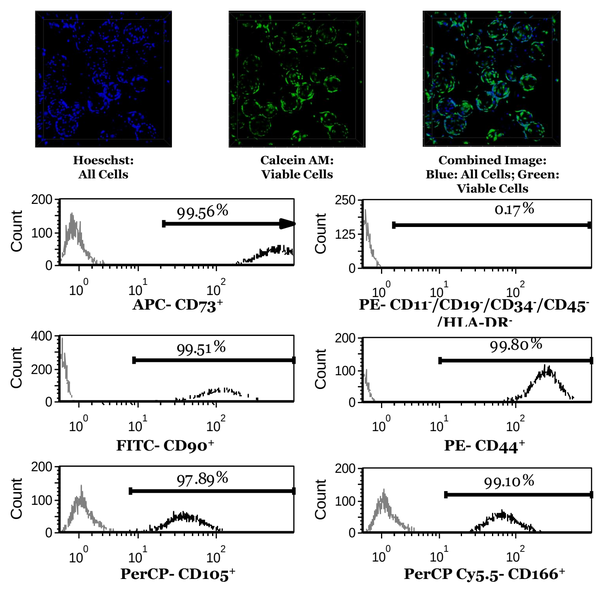


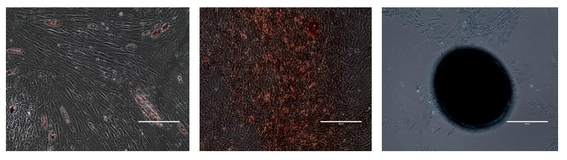
Cell Quality

Microcarriers containing cells were stained during growth and imaged using confocal microscopy, providing a basis for a visual cell distribution assessment.

Phenotypic assessment of the generated cells was performed using flow cytometry to detect the presence of CD44$^+$; CD73$^+$; CD90$^+$; CD105$^+$ and CD166+ at levels ≥95%; the absence of CD11b$^-$; CD19$^-$ CD34$^-$; CD45$^-$ and HLA-DR$^-$ markers at levels ≤2%.

hMSC differentiation potential to the adipogenic, osteogenic and chondrogenic lineages was shown using cell differentiation and staining.





Conclusion

Integration of closed, single-use allogeneic platform process, for the culture and harvest of hMSC cell product was demonstrated. The selection of optimum process options achieved yield increases while maintaining critical quality attributes. Ongoing complete process closure will decrease risk of sterility breach, increasing process robustness and reliability for GMP process application, along with smarter in process controls to ensure successful batch delivery.

We work with Innovate UK

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