PROCESS DEVELOPMENT OF HUMAN MESENCHYMAL STEM CELL MICROCARRIER CULTURE USING AN AUTOMATED HIGH-THROUGHPUT MICROBIOREACTOR

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Improvements to process development technology will have a significant impact in reducing the overall costs associated with the manufacture and scale-up of human cell-based therapies. Single-use, small-scale models, including microbioreactors, play a critical role in this regard as they reduce reagent requirements and can facilitate high-throughput screening of process parameters and culture conditions. Here we have demonstrated, for the first time, the amenability of the automated ambr15 cell culture single-use, microbioreactor system (originally designed for free suspension culture) for adherent hMSC microcarrier culture. We also demonstrated that the ambr15 could be used for bioprocess development of a microcarrier process which was subsequently validated with larger-scale single-use spinner flask studies.

The results were achieved by a combination of strategies including adapting the free suspension design of the vessel to improve the suspension and mixing of the microcarriers. A more effective cell attachment method was also developed by using only 50% of the final working volume of medium for the first 24 h combined with an intermittent agitation strategy. These improvements led to a reduction in the initial lag phase which in turn resulted in > 150 % increase in viable cell density after 24 h compared to the original process (no agitation for 24 h and 100 % working volume). Using the same methodology as in the ambr 15, similar improvements were obtained in larger scale spinner flask studies.

Finally, this improved bioprocess methodology, which was developed for a serum-based medium process, was applied to a serum-free process in the ambr15; this resulted in > 250% increase in yield compared to the ambr15 serum-based process. The use of the ambr15, with its improved control compared to the spinner flask, reduced the coefficient of variation on viable cell density in the serum containing medium from 7.65% to 4.08%, and the switch to the serum free medium further reduced these to 1.06% and 0.54% respectively. The combination of both serum-free and automated processing improved the consistency more than 10-fold compared to the initial manual, serum-based spinner flask work. The findings of this study demonstrate that the ambr15 microbioreactor is an effective tool for bioprocess development of hMSC microcarrier cultures and that a combination of serum-free medium and automation improves both process yield and consistency.

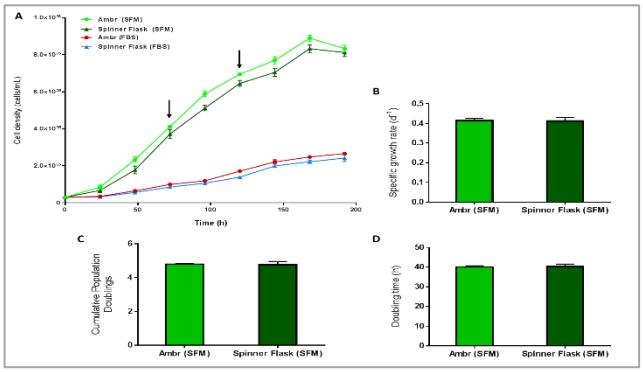

Figure 1 – Growth kinetics of hMSCs for serum-free (SFM) and fetal bovine serum (FBS)-based media in both the ambr15 and spinner flasks with data showing (A) the viable cell density, (B) specific growth rate, (C) the cumulative population doublings and (D) the doubling time. Data show mean ± SD, n = 8.

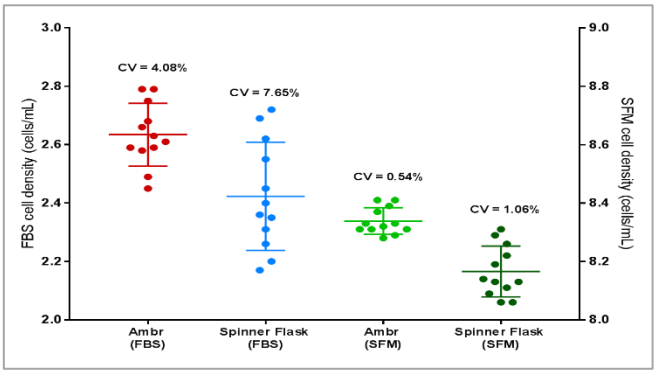

Figure 2 – Extent of viable cell density variation in the ambr15 and spinner flask for both serum-free (SFM) and fetal bovine serum (FBS)-based cultures. Cell density values for FBS are aligned with the left y-axis and the SFM values with the right y-axis. Data show coefficient of variation (CV), n = 8.