INTENSIFIED VIRUS PRODUCTION IN SUSPENSION HEK293SF CELLS USING HIGH INOCULUM FED-BATCH OR LOW-PERFUSION RATE CULTURES WITH CONTINUOUS HARVEST

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Advances in large-scale cell culture techniques have encouraged the development of cell-based processes for influenza vaccine production as an alternative to the current egg-derived production systems. While cell culture offers a valuable alternative, productivities are still low when compared to traditional egg-based systems, requiring extensive efforts in process intensification. Major efforts in the intensification of cell-based viral vaccine manufacturing focus on the development of high cell density processes, often operated in perfusion. While perfusion operations allow for higher viable cell densities and volumetric productivities, the high flow rates normally adopted – typically between 2 and 4 vessel volumes per day (VVD) – dramatically increase media consumption, resulting in a higher burden on the cell retention device and raising challenges for the handling and disposal of high volumes of fluid. High inoculum fed-batch (HIFB) processes and low-perfusion rate processes with an additional concentrated feed reduce the volume of media consumed while sustaining high viable cell densities, and may be applied for stable products when the production is limited by nutrient depletion instead of by-product accumulation. In this study, we explored the use of HIFB and low-perfusion rate operations to intensify a cell culture-based process for influenza virus production. Feeding strategies were initially developed and optimized in 50mL TubeSpin shake-tubes (ST-50mL). Cultures were inoculated at 2 ×10^6 cells/mL and were operated, during the growth phase, either in fed-batch (intermittent addition of concentrated feed) or pseudo-perfusion (partial media exchange with supplemented basal media, equivalent to 0.5 VVD). After high cell density infection with pandemic influenza A/Puerto-Rico/8/34 (H1N1), performed between 8 and 10 ×10^6 cells/mL, cultures from both strategies were semi-continuously harvested at 0.5 VVD. The optimized feeding strategies were then scaled-up and assessed in a 3L bioreactor. Both the perfusion operation and the continuous harvest were performed using a TFDF® module (Repligen), with a perfusion rate of 0.5 VVD. Virus production was evaluated by the total volumetric productivity (HAU/L.day), the yield on media (HAU/L) and the cell specific virus yield (HAU/10^6cells). When evaluated in small-scale, the developed feeding strategies resulted in up to 6-fold increase in volumetric productivity while maintaining similar values of yield on media and cell specific virus yield in comparison to the low-cell-density batch control (infected at 2x10^6cells/mL). The fed-batch strategy was successfully scaled-up to a 3L bioreactor, resulting in productivity and titer comparable to small-scale. However, a decrease in titer and productivity was observed for the perfusion bioreactor culture, most likely due to the inherent differences between the semi-continuous small-scale model and the continuous perfusion operation in the bioreactor. Current efforts aim at optimizing the perfusion operating conditions. Overall, the results show that feeding strategies that minimize media consumption and handling can be successfully applied to intensify virus production processes.

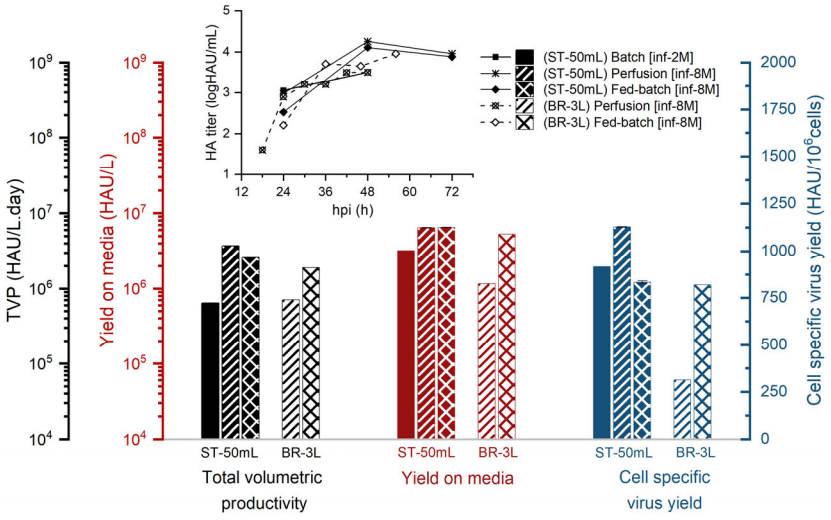

Figure 1 – Virus titer and productivity of two intensified and scalable cell culture processes for influenza virus production in comparison to a standard low-cell-density batch.

ST-50mL = 50mL shake-tubes;
BR-3L = 3L bioreactor equipped with a TFDF® module (Repligen) as cell retention device.