6-12-2022

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INTENSIFICATION OF INFLUENZA VIRUS PRODUCTION IN FED-BATCH AND PERFUSION CULTURES OF HEK293SF CELLS

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Key Words: Virus production, influenza, suspension cell culture, process intensification

More than half a million people die every year from complications of seasonal influenza, and vaccination stands as the most effective method to prevent and limit outbreaks of the disease. Constant vaccine development based on emerging strains and worldwide distribution of vaccines is a great challenge for public health and vaccine manufacturers, particularly in a potential pandemic scenario. The limited flexibility of the current egg-based production system combined with recent advances in large-scale cell culture techniques have encouraged the development of cell culture processes for influenza vaccine production. While cell culture offers a valuable alternative, productivities are still low when compared to traditional egg-based systems, requiring extensive efforts in process intensification and suspension cell line development.
In this study, we explored different process operations to intensify a cell culture-based process for influenza production, while minimizing process complexity. Feeding strategies, applied to standard or high inoculum (HI) cell culture processes, were tested in 50mL TubeSpin shake tubes. During growth phase, suspension HEK293SF cells were either cultivated in fed-batch (intermittent addition of concentrated feed) or pseudo-perfusion (p-perfusion - partial media exchange with supplemented basal media, equivalent to 0.5vvd), and were all shifted to pseudo-perfusion (0.5vvd) after infection with pandemic A/Puerto-Rico/8/34 (H1N1), performed between $5x10^6$cell.mL^{-1} and $12x10^6$cell.mL^{-1}. Two batch controls, infected either at low or high cell density ($1x10^6$cellsmL^{-1} and $4x10^6$cells.mL^{-1}) were defined. Virus production was evaluated by the total yield (TY, expressed in VP), the yield on media (Y/M, expressed in VP.mL^{-1}) and the cell specific virus yield (CSVY, expressed in VP.cell^{-1}). The tested feeding strategies resulted in total yields 1.8-fold to 3.3-fold higher when compared to the most performant batch control. With exception of the *HI p-perfusion/p-perfusion* strategy (high inoculum, operated in p-perfusion during both growth and infection phases), all strategies showed comparable or higher Y/M and CSVY when compared to the most performant batch control. Infection at higher cell densities, performed for HI cultures, resulted in decreased virus production when compared to the same feeding strategy at lower cell densities, indicating a “cell density effect” most likely related to nutrient limitations. These results show that feeding strategies that minimize media consumption and handling, such as low-exchange-rate perfusion and fed-batch, can be successfully applied to intensify virus production processes with little added complexity. Future work includes the improvement of these strategies to avoid nutrient limitations and scale up to benchtop bioreactors.

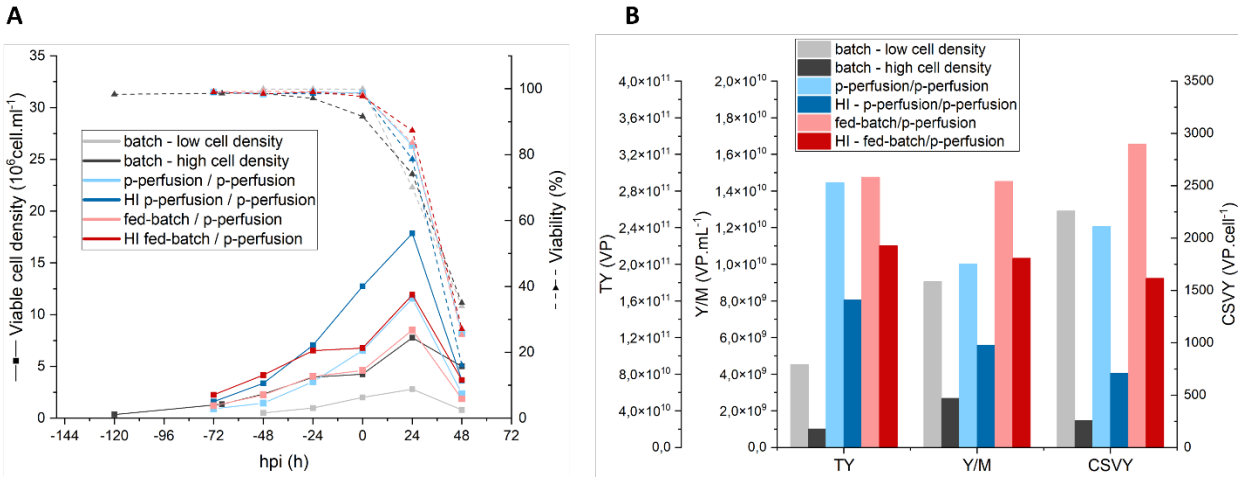

Figure 1 – Cell culture-based production of influenza virus using different feeding strategies. A) Cell growth and viability and B) viral yield and productivity. TY = total yield, Y/M = yield on media, CSVY = cell specific virus yield, VP = viral particles, HI = high inoculum, p-perfusion = pseudo-perfusion. Labels for feeding strategies = feeding-strategy-during-growth-phase / feeding-strategy-at-infection-phase