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MULTIVALENT INFLUENZA VACCINE PRODUCTION IN HEK293SF CELLS IN RESPONSE TO PANDEMIC THREATS

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Influenza virus infects millions of people every year worldwide, with elderly and very young people among the most critically affected. Strains that constitute a pandemic threat are characterized by the severity of the clinical manifestations and mortality rates and tend to require the urgent production of hundreds of millions of vaccine doses in very short periods of time. There is an evident need to develop new generations of influenza vaccines based on robust production systems such as mammalian or insect cell cultures. These systems may allow, in contrast to production in embryonated chicken eggs, a faster response capacity, a superior manufacturing process control and a more reliable and better characterized product.

In this work, we have used influenza strains that constitute a potential pandemic threat (H1N1, H3N2 and H7N9 subtypes) for the development and evaluation of a vaccine production process in HEK293SF cells. The analysis of culture parameters was conducted in parallel using the A/PR/8/34 (H1N1) and the A/Hong-Kong/8/68 (H3N2) strains plus one H7N9 subtype reassortant. The reassortant virus carrying the HA and NA genes of the A/Anhui/1/2013 strain on a PR8 backbone was obtained and amplified in HEK293SF cells using an 8 plasmid-based reverse genetics technique followed by an adaptation step in HEK293SF. HycellTransFx-H and Xell HEK-GM serum-free media were evaluated for virus production in shake flask experiments. A range of culture parameters was evaluated in batch including different cell densities at infection; infection at different MOI; temperature shift at the time of infection and trypsin addition to the medium at different concentrations. Process intensification using fed-batch and perfusion culture modes were also evaluated.

Virus yields obtained in batch with HEK-GM medium were more than one log lower than those obtained with HycellTransFx-H. Also, the implementation of a fed-batch mode was able to overcome medium limitation at higher cell densities. Perfusion was a more effective strategy allowing a final virus yield increment of about 2 logs. Culture conditions were scaled-up and ten controlled batch and fed-batch bioreactor runs (1-3 liters) were completed using HycellTransFx-H, aiming to gain deeper insights into requirements for a robust upstream bioprocess, generate material for downstream development and optimize analytical techniques for virus particles quantification. On-line monitoring and reproducibility of the production kinetics was also followed in the 3L runs by capacitance measurements. Median Tissue Culture Infectious Dose (TCID50)/mL in the range of 10^9 IVP/ml was obtained for the H1N1 strain while values over 10^7 and 8 x 10^7 IVP/ml were achieved for the H7N9 and H3N2 subtypes, respectively. The quantification methods used comprised $TCID_{50}$/mL (IVP/per mL), viral genomes quantified by ddPCR (copies/ml), hemagglutination (HA units/ml) and neuraminidase (NA units/ml) assays, as well as dot blot and SRID (both ug of HA/ml).

In summary, this work is a further step providing solid bases for the completion of a scalable and robust manufacturing process for a multivalent influenza vaccine in HEK293SF cells, process that might be activated and scaled-out in response to influenza pandemic situation.