6-12-2022

Development of scalable downstream processing platform for HEK293SF cell-based influenza vaccine production

Amine Kamen

Xingge Xu

Zeyu Yang

Irene González Domínguez

Cécile Toussaint

See next page for additional authors

Authors

Amine Kamen, Xingge Xu, Zeyu Yang, Irene González Domínguez, Cécile Toussaint, Alice Bernier, Alina Venereo Sanchez, Omar Famos, and Cristina Aglaia Alves Tottoli e Silva

DEVELOPMENT OF SCALABLE DOWNSTREAM PROCESSING PLATFORM FOR HEK293SF CELL-BASED INFLUENZA VACCINE PRODUCTION

Xingge Xu, Department of Bioengineering, McGill University, Canada
Zeyu Yang, Department of Bioengineering, McGill University, Canada
Irene González Domínguez, Universitat Autònoma de Barcelona, Spain
Cécile Toussaint, Department of Bioengineering, McGill University, Canada
Alice Bernier, Department of Bioengineering, McGill University, Canada
Alina Venereo Sanchez, Department of Bioengineering, McGill University, Canada
Omar Farnos, Department of Bioengineering, McGill University, Canada
Cristina Aglaia Alves Tottoli e Silva, Department of Bioengineering, McGill University, Canada
Amine A. Kamen, Department of Bioengineering, McGill University, Canada

Key Words: influenza vaccine, HEK293 cells, Anion exchange chromatography, large-scale purification, centrifugation-free downstream processing.

Background: Research efforts during recent decades have demonstrated the suitability of mammalian cell culture platform for influenza vaccine production. Certainly, the potential of this system for a large-scale continuous vaccine manufacturing will enable a faster response to pandemic comparing with traditional egg-based production. Even though great advances have been achieved on the upstream processing of mammalian cell culture produced influenza vaccines, the downstream processing and quality of final product have still room for improvement or is still in development.

Method: In this study, we propose a scalable filtration/chromatography-based purification process consisting of large scale-like clarification steps including Benzonase® treatment, cell sedimentation followed by depth filtration, and inactivation with beta-propiolactone. The whole-inactivated virus in supernatant is captured by an ion-exchange chromatography and eluted at high salt concentration in the presence of 0.005% (w:v) Zwittergent 3-14 followed by buffer exchange.

Result: The downstream processing was implemented using three pandemic influenza virus A/PR/8/34 (H1N1), A/Hong-Kong/8/68 (H3N2), and one H7N9 subtype reassortant from batch bioreactor productions in HEK-293SF cells. With Benzonase® digestion and clarification using a depth filter we can achieve more than 90% reduction in dsDNA while still recovering 70% to 80% hemagglutinin, which is comparable with clarification by centrifugation. The results are consistent for the three virus strains we studied. Three ion exchange membranes, NatriFlo® HD Q, Mustang® Q, and Sartobind® Q were evaluated in the chromatography step. Results of experiments on influenza A/PR/8/34 H1N1 indicate that the Mustang® Q is the optimal membrane which can achieve the best DNA removal at 97%, being eluted from the membrane with 700 mM NaCl in 50 mM HEPES, 2mM $MgCl_2$, 0.005% (w:v) Zwittergent 3-14 at pH 7.5, with a corresponding 40-50% in HA recovery. Sartobind® Q shows a slightly better recovery of HA than Mustang® Q under the same eluting condition but with less DNA removal. Moreover, the same downstream process steps under similar operating conditions were performed with H7N9 and H3N2， and got hemagglutination units recoveries of 40.1% and 47%, respectively, indicating a generic platform for purification of pandemic influenza strains.

Conclusion: This membrane chromatography-based centrifuge-free purification process is promising to complete a fully large-scale compatible manufacturing process for cell-produced multivalent influenza vaccines.