PRODUCTION OF BIOPHARMACEUTICALS IN AN INTENSIFIED PERFUSION PROCESS OF HEK 293 CELLS

Johan Rockberg, KTH ; Wallenberg Centre for Protein Research (WCPR) ; NovoNordisk Center for Biosustainability (NNF CFB), Sweden
Caijuan Zhan, KTH ; Wallenberg Centre for Protein Research (WCPR)
Magdalena Malm, Wallenberg Centre for Protein Research (WCPR)
Hubert Schwarz, KTH ; Wallenberg Centre for Protein Research (WCPR)
Magnus Lundqvist, KTH ; Wallenberg Centre for Protein Research (WCPR) ; NovoNordisk Center for Biosustainability (NNF CFB), , Sweden
Atefeh Shokri, KTH ; Wallenberg Centre for Protein Research (WCPR) ; NovoNordisk Center for Biosustainability (NNF CFB), , Sweden
Ray Field, BioPharmaceutical Development, MedImmune, Cambridge, UK
Richard Turner, BioPharmaceutical Development, MedImmune, Cambridge, UK
Veronique Chotteau, KTH ; Wallenberg Centre for Protein Research (WCPR) ; AdBIOPRO, Competence Centre for Advanced Bioproduction by Continuous Processing

CHO cells are the workhorses of the biopharmaceutical field with many success stories. However human cell-based systems might bring important advantages. These can provide production systems resulting in proteins with more human-like posttranslational modifications and potentially alleviate the production of difficult-to-produce molecules. HEK 293 cells are well known and used today for the production of two biopharmaceuticals and for viral vectors. The purpose of the present study is to evaluate the potential of this system for its ability to produce biopharmaceuticals, benchmarking against CHO cells.

We are currently exploring the possibility to secrete human proteins in CHO cells by systematically addressing all the human proteins naturally secreted in the human body. So far, we have covered around half of the human secretome (N = 3000) with an overall success rate around 65%. To address the need for a host capable of expressing difficult-to-produce proteins, different HEK 293 strains have been investigated for the production of 30 selected proteins in comparison with CHO cells, revealing a higher success rate in HEK 293 system. This expression has been studied in flask system and includes comparative transcriptomics analyses.

To evaluate the potential of HEK 293 cells for the production of biopharmaceuticals, a high cell density perfusion process using Alternating Tangential Flow filtration has been developed for the production of EPO. In this process, the cells are stably maintained at a density of 80 to 100 x 10^6 cells/mL while the EPO cell specific productivity is comparable to low cell density (e.g. 20 x10^6 cells/ml) in perfusion mode. The cell metabolism is slowed down by lowering the temperature, allowing a reduction of the perfusion rate down to 1 reactor volume per day at this high cell concentration. This process has been developed in our new scale-down perfusion bioreactor of 200 mL working volume. In this system, the effect of shear stress on the HEK 293 cells resulting from their passage in the hollow fibre filter has been characterised by transcriptomics analysis helping to decipher why HEK 293 cells are more sensitive than CHO cells and a systematic feeding strategy for perfusion has been developed.

The ability to express difficult-to-produce proteins and to achieve very high cell densities with productivity comparable to low density processes make HEK 293 cells an attractive system for the production of biopharmaceuticals which are challenging for CHO cells.