Long term cultivation of a protein secreting HEK293 cell line in 3 parallel self-contained hollow fiber bioreactor systems

Dr. Jana Langenhan[1], Dr. Hans Nagels[2]

[1] EUROIMMUN Medizinische Labordiagnostika AG, Werkstraße 22, 23942 Dassow, Germany
[2] Cellab GmbH, Juri-Gagarin Straße 13a, 01454 Radeberg, Germany

Experimental goal

A stable variant of a HEK293 cell line was used, which after induction with Doxycycline expresses the target protein and secretes it into the medium. The conformation of the protein is very unstable so it cannot be produced in typical serum-free or chemically defined media (e.g. for suspension culture). For large scale production only adherent culturing on a large surface area is possible.

In this experiment the HEK 293 cells were cultured in a hollow fiber (HF) based cultivation system, the Cellab® Bioreactor System from Cellab GmbH. The Cellab® Disposable Set 5 HF/S allows culturing of cells in parallel reactors and adjusting different culture parameters during the experiment. This represents a flexible downscaling model for the upcoming large scale production of the target protein.

The HF module itself is composed of hundreds of tube-like membranes with a huge surface-to-volume ratio. These semipermeable membranes form a two-compartment system that separates the cell side from the medium side (see fig. 1). In this way the ongoing supply of nutrients and the removal of metabolites can be achieved. At the same time the volume of the cell compartment can be minimized. As a consequence a high cell density accompanied with a high target protein concentration can be obtained.

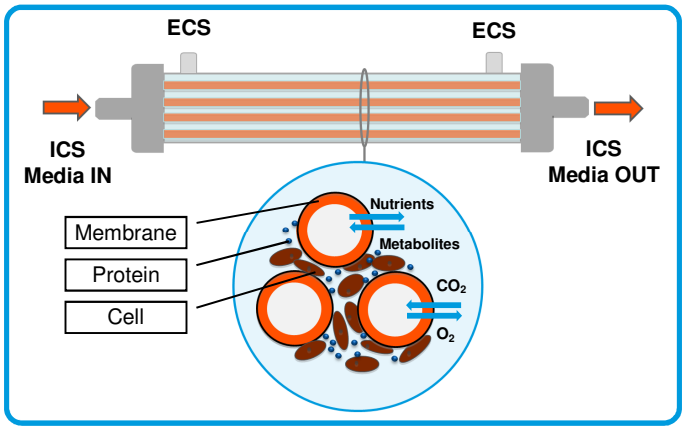

Fig.1: Scheme of a cross section through a hollow fiber module and cultivation of cells in the ECS

The HF modules and the gas transfer modules, used to ensure the necessary gas exchange in a sterile manner, are integrated in the self-contained Cellab® Disposable Set and are operated on the Cellab® Docking Station in a standard 37°C CO_2-incubator. A semi-automated process controlled by the docking station regulates the continuous supply of nutrients and oxygen for the cell cultivation.

Material and methods

The experiment was carried out with a Cellab® Disposable Set 5 HF/S (Cat. No. 0510026) operated by the Cellab® Docking Station with a 5-channel media pump (Cat. No. 0510017) and with a flow rate set at 4.6 ml/min. The bioreactor membrane is made of polysulfone and has a MWCO of 20 KDa. The extra capillary space (ECS) culturing surface of each module is 190 cm^2 and the corresponding volume of the ECS is 1.8 ml. HEK293 cells were cultivated in the ECS of 3 hollow fiber bioreactors in DMEM High Glucose with 1 x Penicillin/Streptomycin and 10 % initial FCS (fetal calf serum) concentration. The remaining 2 HF modules in the 5 modules set were not used in this experiment as the experimental design was structured for 3 parallel cell cultures.

The reservoir medium, which is pumped through the intra capillary space (ICS), contained DMEM High Glucose with 1 x Penicillin/Streptomycin as well, but without FCS. Furthermore 2 µg/ml Doxycycline were added to the medium to induce protein expression.

On day 1 the ECS compartment of each 3 hollow fiber module was inoculated with $2x10^6$ cells. This cell concentration seemed not sufficient for a viable inoculation as measured by the glucose level which did not decrease during the first days of the culture. Cell inoculation was repeated on day 7 with $2x10^7$ cells per HF module, which led to detectable glucose consumption within a few days.

The medium in the reservoir was changed two to three times per week using a sterile 50 ml syringe and the 3-way stopcock located in the tube assembly behind the HF bioreactors. Each of the 3 HF modules consumed 200-400 ml of medium per week. Sampling was done with a sterile 1 ml syringe using the same 3-way stopcock. The glucose concentration was measured with a GlucCell Glucose Monitoring Kit (Cesco). The target protein concentration was determined with StainFree gels (BioRad) over a Geldoc EZ with the related Software Image Lab.

Results

The glucose consumption of the 3 hollow fiber bioreactor modules (see fig. 2) was adapted over a period of 65 days to an average value of 135 mg/day. Over the culture period the 3 bioreactor modules showed a high level of comparability which demonstrates the excellent reproducibility of the system.

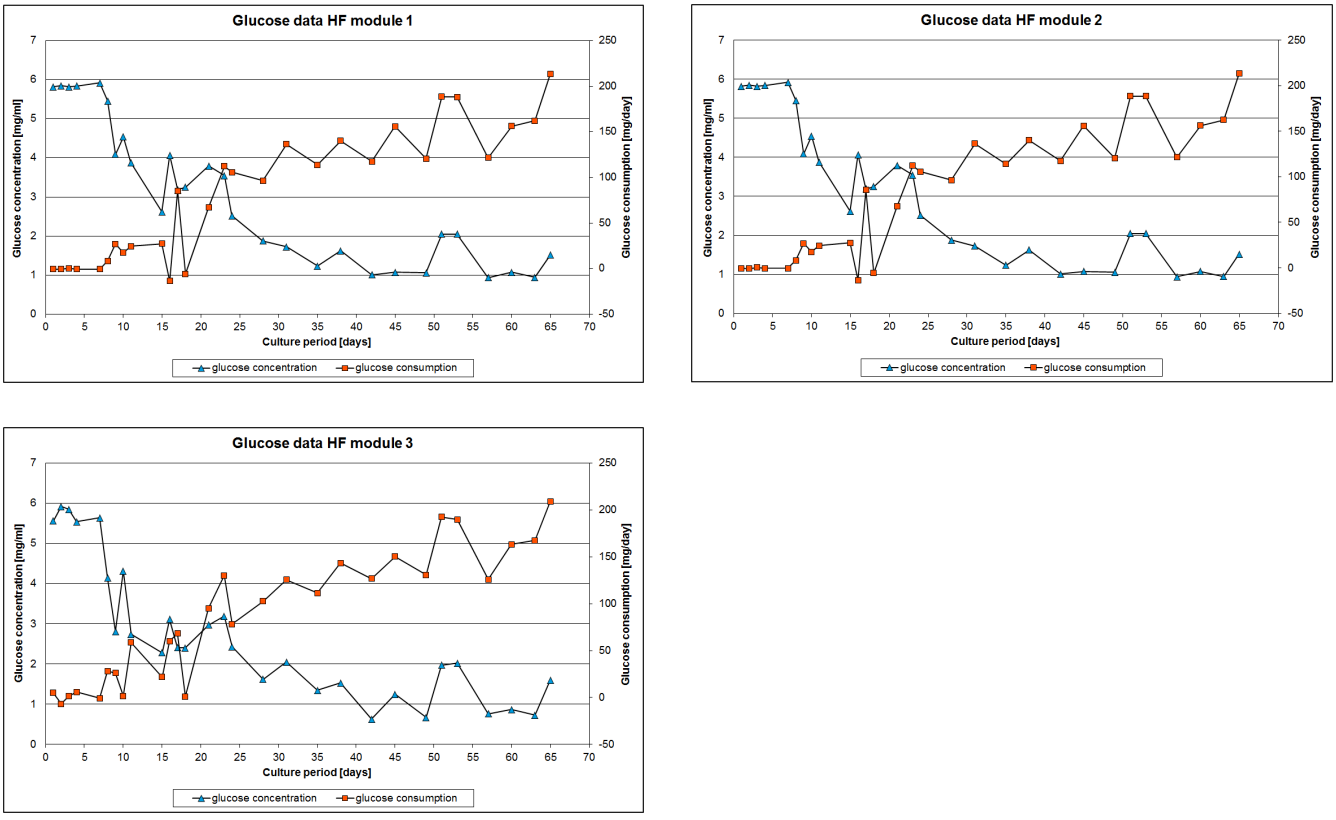

Fig.2: Glucose consumption of the 3 hollow fiber bioreactors over a culture period of 65 days

The secreted protein of the HEK293 cells, which is visible at 100 kDa in the gel (see mark in fig. 3, 4, 5), can be easily harvested from the ECS of the HF bioreactor using a sterile 2 ml syringe. With each harvest of 1.6 ml some of the cells are flushed out. This prevents having a too high cell density in the module and allows continuous protein production. The harvest volume was then centrifuged to achieve a cell free product from the supernatant.

Over an extended period of time the calculated mass concentration of the target protein remained stable in each module under the same cultivation conditions. Between the HF modules 1, 2 and 3 there is no significant difference detectable when working under identical culture conditions.

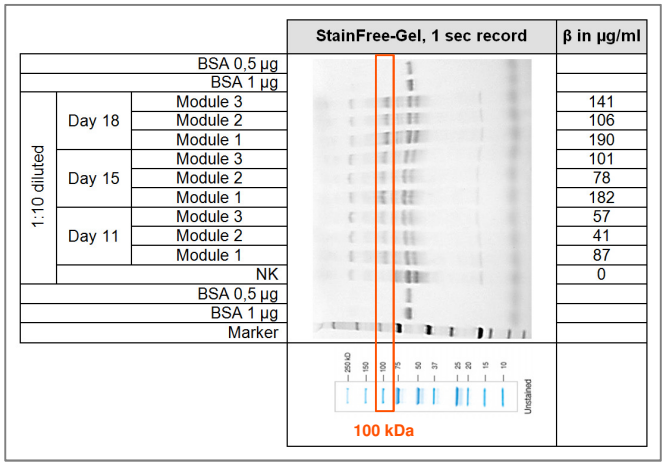

Fig.3: StainFree-Gel, applied are cell culture supernatants from the ECS of the 3 bioreactors on day 11, 15 and 18 and the protein concentration measured in µg/ml

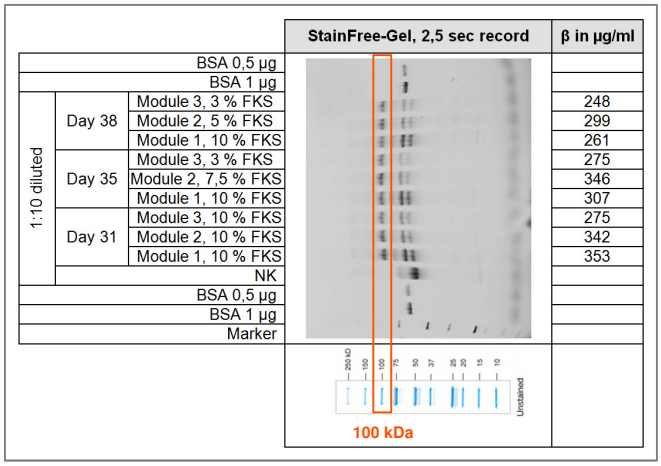

Fig.4: StainFree-Gel, applied are cell culture supernatants from the ECS of the 3 bioreactors on day 31, 35 and 38 and the protein concentration measured in µg/ml under reduced FCS concentration in the ECS medium

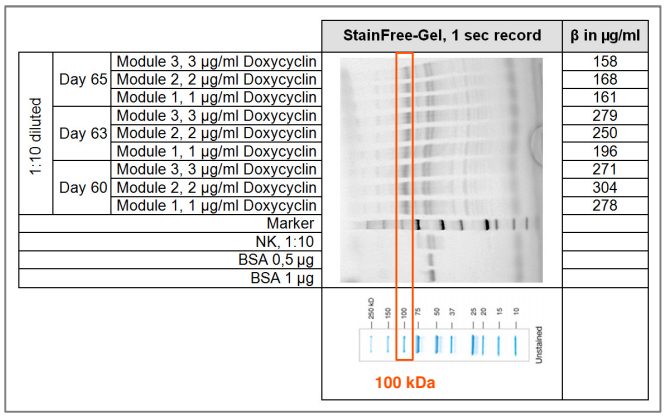

Fig.5: StainFree-Gel, applied are cell culture supernatants from the ECS of the 3 bioreactors on day 60, 63 and 65 and the protein concentration measured in µg/ml with adjusted concentrations of the induction antibiotic Doxycycline in the ICS and ECS medium

From day 28 onwards the FCS concentration in the ECS medium compartment in module 2 was reduced from 10 % to 3.5 %. This led to a slight decrease of protein concentration (see fig. 6). An abrupt change from 10 % to 3 % FCS is also possible, which was performed in module 3.

This reduction of the FCS led to a substantial improvement of the purification of the target protein as a result of less foreign proteins in the native sample. Despite a lower protein yield, this resulted a significant cost reduction for the overall production process.

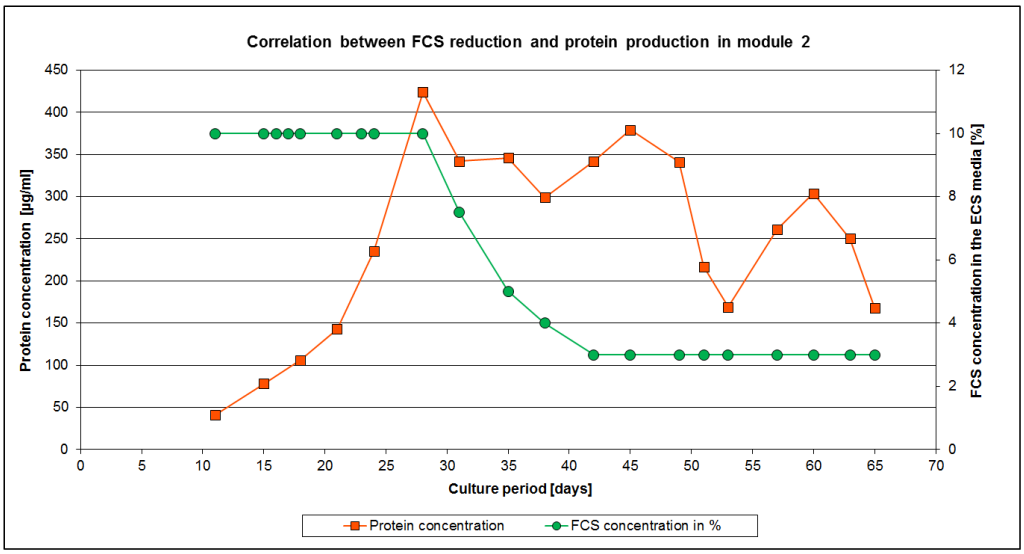

Fig.6: Correlation between FCS reduction and protein production in module 2

Conclusion:

The long-term cultivation of protein secreting HEK293 cells in hollow fiber based bioreactors over a period of 65 days demonstrated the efficiency of this culturing method. The protein concentration of the harvest fraction reached up to 500 µg/ml. The cultivation pattern of the 3 hollow fiber bioreactors under the same culture parameters was nearly identical, which can be clearly observed in the glucose consumption (see fig. 2) and the protein concentration (see fig. 7).

This demonstrates that the Cellab® Disposable Set 5 HF/S is ideally suited for the validation of production processes in lab scale under reproducible culture conditions.

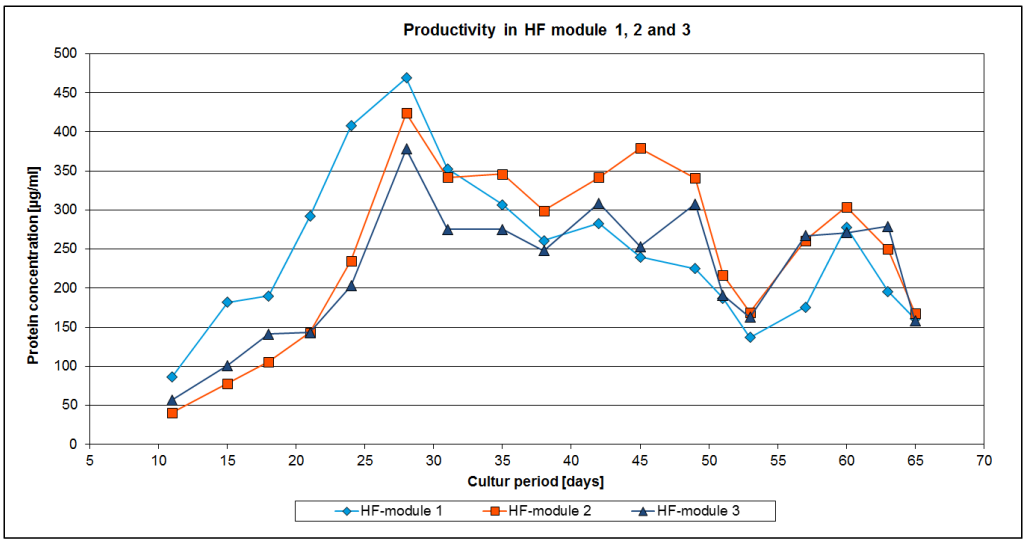

Fig.7: Protein productivity in 3 hollow fiber modules over a period of 65 days