Characterisation of cultivation of the human cell line AGE1.HN

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Introduction

Human cell lines are an interesting alternative to CHO cells for the production of recombinant proteins and monoclonal antibodies, because of their ability to produce genuine human posttranslational modifications. The human cell line AGE1.HN.AAT (ProBioGen, Berlin, Germany), that originated from human neural precursor tissue, has been adapted to serum-free conditions and cultivated in many different systems. Here we present our results using this cell line in a scale-up of batch cultivation from 50 mL vented polypropylene tube on a shaking platform, polycarbonate shakeflask (cultivation volume from 50 mL up to 300 mL), a 2 L-glass vessel stirred tank reactor and a 20 L- stainless steel stirred tank reactor (both Sartorius Stedim, Goettingen, Germany).

Methods

Cultivations were performed with chemically-defined and animal-component-free media 42-MAX-UB (Teutocell, Bielefeld, Germany). Batch-cultivations were performed in 50 mL bioreactor tubes (TPP, Switzerland) shakeflasks (Corning Life Sciences, Netherlands), 2 L-glass vessel and 20 L-stainless steel vessel (both Sartorius-Stedim, Germany). Chemostat-cultivation was done in 0.5 L-bioreactor (DASGIP, Juelich, Germany) with a media exchange rate of 7 mL/h, resulting in constant cell growth over two weeks with a nearly constant viable cell density of 2.5 E6 cells per milliliter. As a further cultivation system a dialysis-reactor (Bioengineering, Wald, Switzerland) was established, with a 1.3 L cell-containing inner chamber and a 4 L media reservoir in the outer chamber, separated by a semipermeable dialysis membrane. Batch cultivation without media-exchange in the outer chamber showed maximum cell density up to 1.6 E7 viable cells per milliliter in the inner chamber.

Results

Proliferation in uncontrolled systems:

The AGE1.HN.AAT cells show similar growth in different unregulated vessels and culture volumes. The used systems ranged from 50 mL bioreactor tubes with a culture volume of up to 18 mL, 125 mL shakeflasks with a culture volume of up to 50 mL to 250 mL shakeflasks, in which a culture volumen of 100 mL can be used.

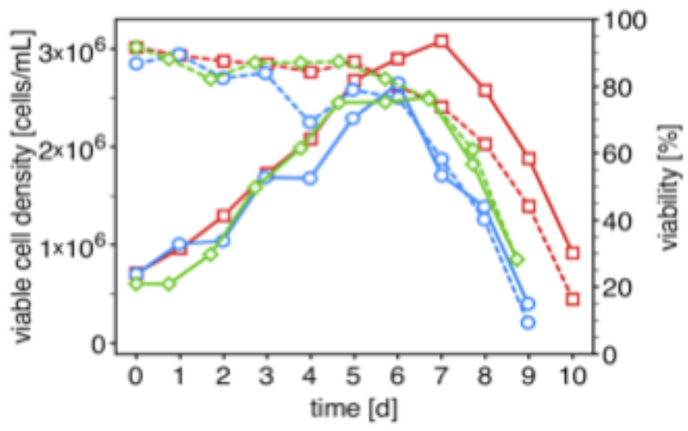

Fig. 1: viable cell density and viability during shake flask batch-cultivation. red: bioreactor tube, blue: 125 mL shakeflask, green: 250 mL shakeflask

Proliferation in regulated systems:

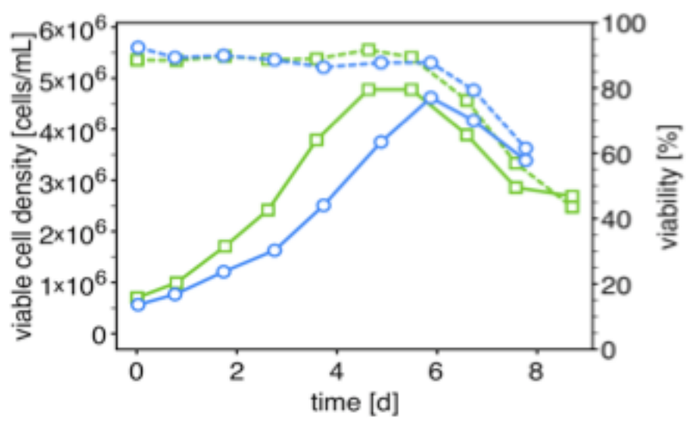

Fig. 2: viable cell densityand viability during bioreactor batch-cultivation. green: 2 L glass-vessel, blue: 20 L stainless-steel reactor

Cultivation at a 20 L-scale resulted in delayed cell growth but did noch affect the final cell concentration. AGE1.HN cells show a strong growth-coupled productivity as shown in Figure 3.

Scale up-effects:

No difference between 2 L- and 20 L-vessel concerning spec. productivity and spec. growth rate were observed.

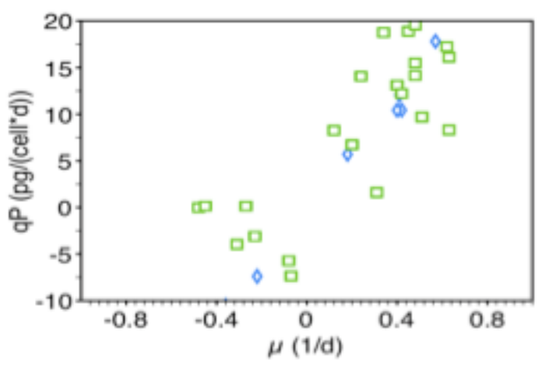

Fig. 3: specific growth rate μ of bioreactor cultivation vs. corresponding specific productivity qP. blue: 20 L-stainless steel reactor, green: 2 L-glass vessel

Other process strategies:

Cultivation in dialysis reactor:

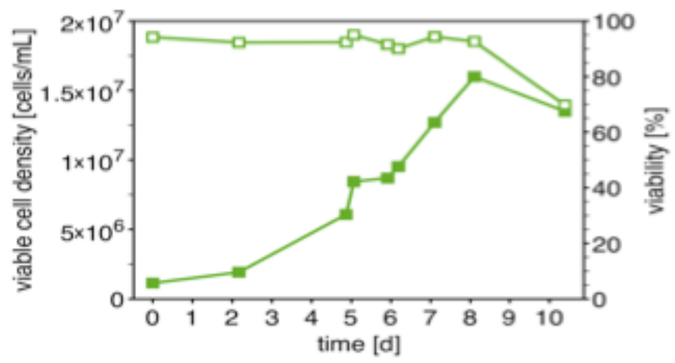

Fig. 4: Viable cell density and viability during cultivation in dialysis-reactor

Cultivation of AGE1.HN cells in dialysis-reactor shows extended maximal viable cell density (up to 1.6 E7 cells/mL).

Chemostat-cultivation:

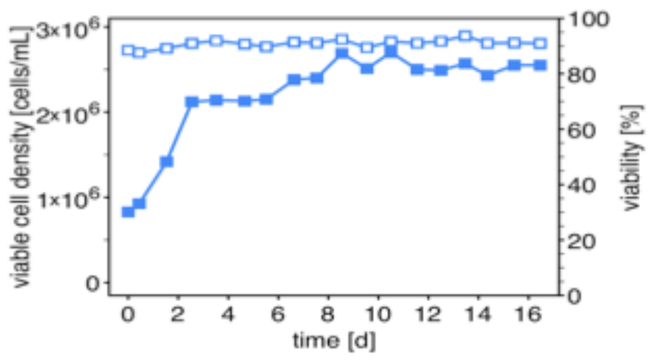

Fig. 5: Viable cell density and viability during chemostat-cultivation.

Chemostat-cultivation shows constant cell density.

Conclusions

- Cultivation of AGE1.HN cell line is possible in different regulated and unregulated systems and at different scales.
- Cell specific productivity and titer of the AGE1.HN.AAT producer cell line depend mainly on cell growth and are easily scalable from 2-20 L batch cultivation in STR.
- Other cultivation strategies have been established successfully (incl. chemostat and dialysis-bioreactor) documenting the potential of the AGE1.HN cell line.

The work presented in this poster was part of SysLogics (Systems biology of cell culture for biologics), which is funded by German Federal Ministry of Education and Research. Cultivation of 125 mL shakeflasks and bioreactor tubes were done in Saarbruecken, dialysis cultivation in Hamburg, chemostat-process was done in Magdeburg, while 250 mL shakeflask, 2 L-glass vessel and 20 L-stainless steel bioreactor-cultivation were performed in Bielefeld.

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