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Overcoming the media design challenges in transient gene expression with CHO cell lines

In this work, the progress in designing special application media for transfection in CHO cells is presented. While transient gene expression (TGE) is routinely performed in HEK cell lines, high transfection of CHO cell lines proves to be somewhat more complex. For modern TGE procedures, a one-step solution and scalability is mandatory. Media components such as iron, which is essential for culture growth, chelators, etc. can inhibit polyethylenimine-mediated transfection, while some polymers have shown to enhance efficiency in e.g. electroporation. In this study, various media components were investigated to facilitate efficient TGE with CHO.

RESULTS

A. Impact of iron components, polymers and chelators on transfection

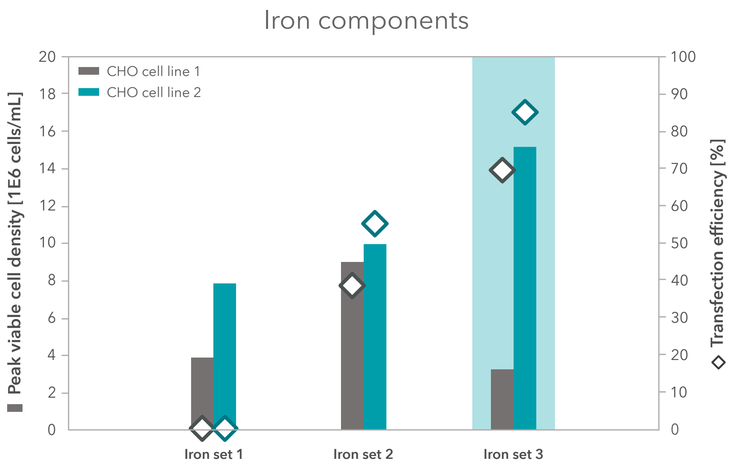

FIG. 1: Iron components in cell culture media are neccessary for cell growth but are known to inhibit transfection[1]. Reducing overall iron concentration and excluding certain iron components helped circumvent this issue. Starting from set 1 (containing mainly iron(II) sulfate heptahydrate) and set 2 (mainly ammonium iron(III) sulfatedodeca-hydrate), iron set 3 was optimized to support transfection.

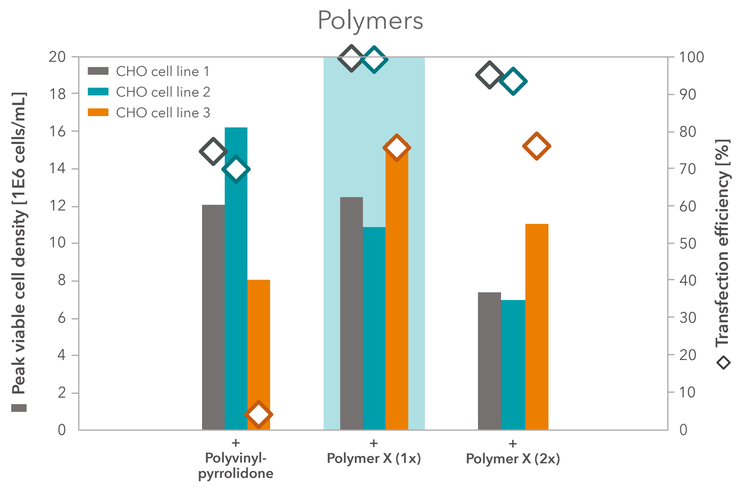

FIG. 2: Polymers such as Polyvinylpyrrolidone (PVP) are known to enhance transfection by e.g. electroporation[2,3]. Results from polyethylenimine (PEI) transfection do not support this effect but another tested polymer (X) was found to increase transfection efficiency for various CHO cell lines. This effect was concentration-dependent and increasing concentrations did not further enhance efficiency.

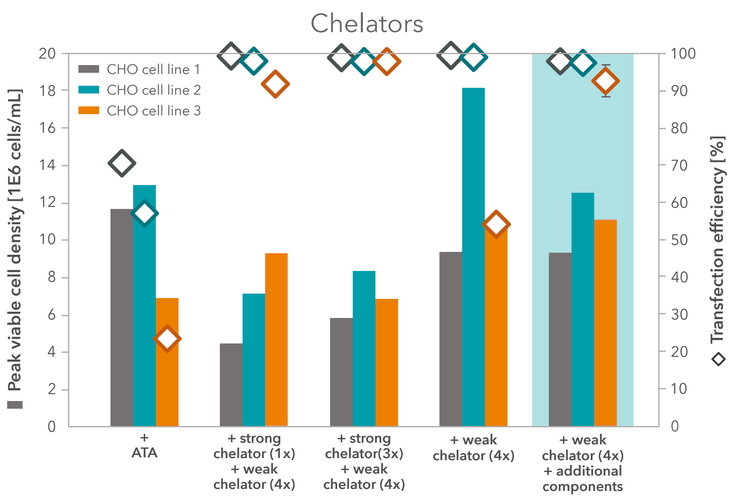

FIG. 3: Aurintricarboxylic acid (ATA) is an endonuclease inhibitor and was reported to enhance transfection e.g. in tissue culture[4]. For the application in CHO suspension culture and PEI transfection, a negative impact of ATA on transfection efficiency was detected. Other strong and weak chelators were inverstigated as well. The strong chelator increasased transfection but impaired growth performance in batch shaking flask cultivation.

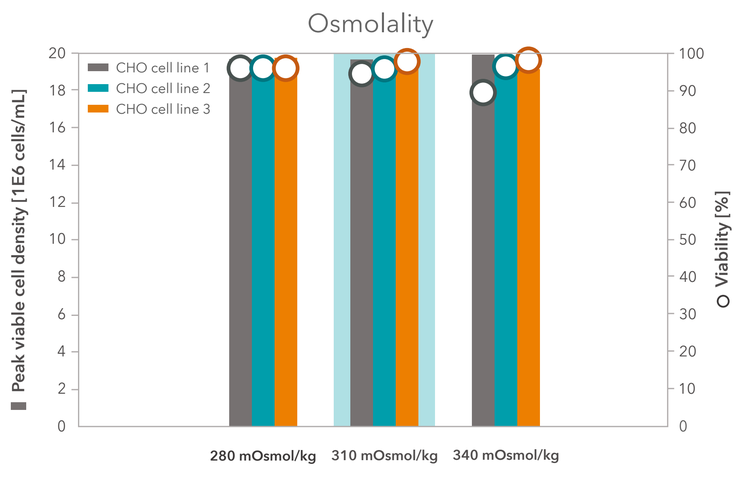

FIG. 4: The effect of osmolality (280-340 mOsmol/kg) was investigated in the final CHO TF medium formulation. In this context, transfection efficiency and viability 48 h post-transfection revealed no major impact in the tested range of osmolality. 310 mOsmol/kg were chosen to account for possible reduction of osmolality during batch production.

B. Growth and transient protein expression in final medium

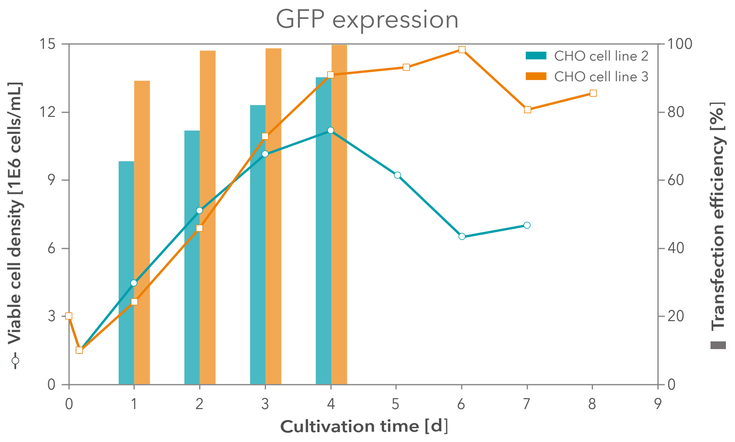

FIG. 5: Growth curves and transfection efficiency of CHO cell line 1 (grey) and CHO cell line 3 (orange) in the final CHO TF medium formulation. Transfection efficiency in CHO cell line 3 was above 90 % from day 2 post-transfection onwards while in CHO cell line 2 the ratio of GFP-expressing cells still increased up to the measurement on day 4.

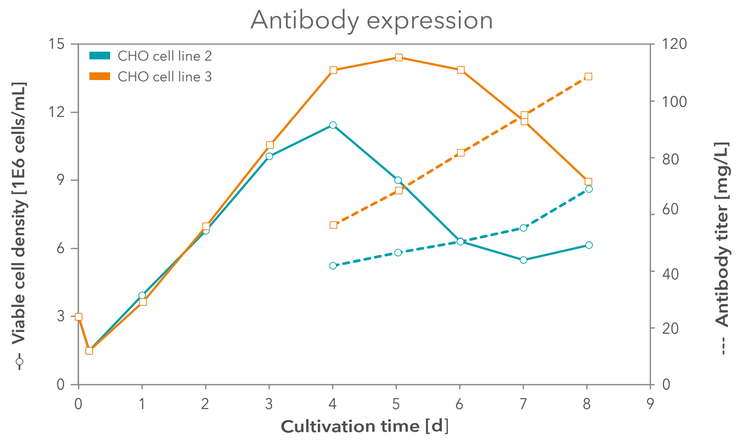

FIG. 6: Cultivations of CHO cell line 2 (grey) and CHO cell line 3 (orange) expressing a monoclonal antibody (mAb) in the final CHO TF medium formulation. Titers were measured from day 4 post-transfection onwards and reached 70 mg/L and 110 mg/L, respectively. For transfection, a simple protocol without the use of feeds, temperature shift or typically used enhancers was applied.

CONCLUSIONS

- The iron composition required careful optimization to support high transfection and sufficient growth. While iron(III) sulfate hepta-hydrate completely prevented transfection, ammonium iron(III) sulfate dodecahydrate still allowed 40-60 % transfection efficiency.
- Addition of PVP did not enhance transfection for the tested CHO suspension cell lines but another polymer (X) increased efficiency when included in the medium at its optimal concentration.
- ATA did not enhance transfection at the tested concentrations. A strong chelator led to high transfection efficiency but negatively impaired cell density in batch processes for various tested concentrations.
- To achieve high transfection efficiency above 90 % while maintaining a peak viable cell density of about $10 \cdot 10^6$ cells/mL in batch processes, a weak chelator and some additional components (including e.g. vitamins) were included in the final formulation.
- Osmolality between 280 mOsmol/kg and 340 mOsmol/kg is generally feasible in the final formulation and no major impact was seen on transfection efficiency or viability 2 d post-transfection.
- Results are based on a simple transfection protocol without pre-complexing of DNA-PEI. Further improvements of the process can be achieved by adapting the protocol.
- The final formulation of CHO TF medium consistently supports high cell growth for tested CHO cell lines with a peak viable cell density above $10 \cdot 10^6$ cells/mL in batch cultivations lasting 7-8 d.
- Transfection efficiency was above 90 % 2-4 d post-transfection in transient GFP expression.
- Growth performance and transient mAb expression was similar to GFP experiments. Using a simple protocol for transfection, titers differed between cell lines with 70 mg/L in CHO cell line 2 and 110 mg/L in the CHO cell line 3.
- Higher yields can be expected through using feeds, a temperature shift or commonly used enhancers, such as valproic acid (VPA).

METHODS

Cultivation All CHO cell lines were cultivated in plain shaking flasks or tube spin bioreactors using standard conditions in chemically defined CHO TF medium or precursors. Growth performance of medium variants was evaluated in batch shaking flask processes.

Analytics Viable cell density and viability were measured using a Cedex automated cell counter. Transfection efficiency was determined via detection of GFP expressing cells in flow cytometry 48 h post-transfection. Antibody yield was measured by protein A HPLC.

Transfection Cells were transfected at densities of $3 \cdot 10^6$ cells/mL in Xell media with PEI-MAX (40.000 MW) and 0.67 pg DNA/cell. DNA was either pCMV-GFP or two plasmids harbouring genes for the expression of a monoclonal antibody (IgG). Culture volumes during transfection ranged from 4 mL to 10 mL in plain shake flasks and tube spin bioreactors shaken at 185 rpm.

LITERATURE

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