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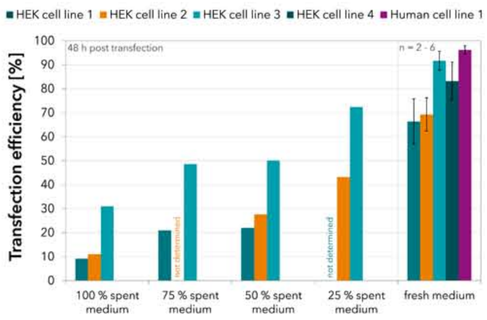
Towards a single chemically defined medium for combining transfection and cultivation of HEK and CHO cell lines

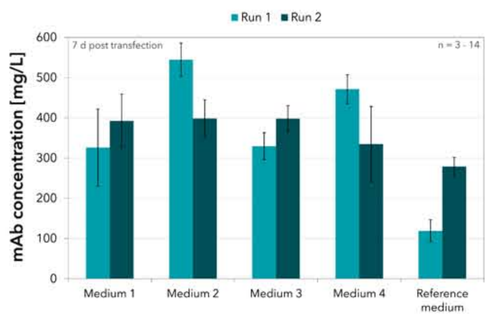
In this work, the current progress in developing a one-stop medium for cultivation and transfection is presented. The significance of transient gene expression in mammalian cells is constantly growing with applications ranging from purposes in R&D to the production of preclinical material. Up to now, challenges for upscaling lie in low transfection efficiencies caused by the presence of spent medium and inefficient cell growth in the transfection medium. To avoid a medium exchange prior to transfection, a bifunctional solution supporting both transfection and cell growth for high productivity is required.

RESULTS - HEK cell lines

1. Transfection efficiencies and productivity

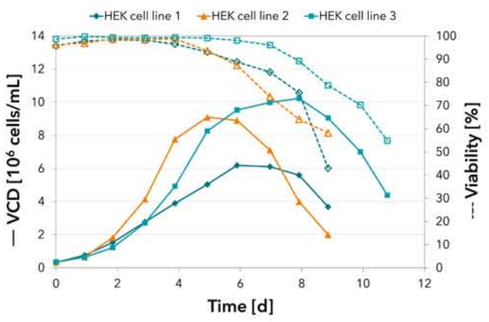


Four different transfection media (1 - 4) are under development. The transfection efficiency in medium 3 is shown for five HEK/human cell lines 48 hours after transfection in fresh medium (multiple independent experiments). Furthermore, the impact of different amounts of spent medium was investigated for three HEK cell lines.

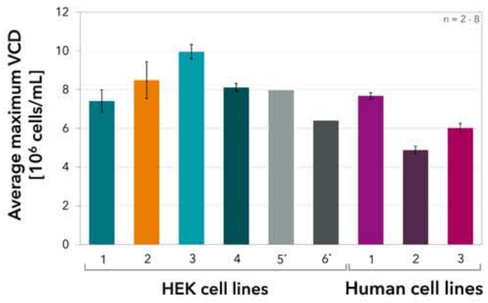


Transient expression of a monoclonal antibody measured in several independent experiments with differing setups using HEK cell line 3 and the developed HEK media 1 - 4. Titers were determined seven days post transfection and compared to results obtained with a commercially available reference medium suitable for growth and transfection.

2. Cell growth in developed media



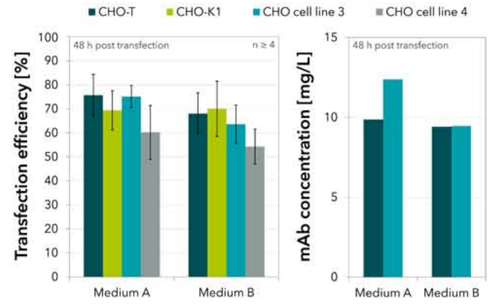
Cell growth in developed HEK transfection media 1 - 4 was evaluated for different HEK cell lines. Cell growth was confirmed to be stable in for more than 70 days during routine passaging and was analyzed in batch shaking flask cultivations. Medium 3 supported growth for more than eight days and cell densities up to $1 \cdot 10^7$ cells/mL.



For six HEK and three additional human cell lines, the maximum viable cell density was determined in batch shaker cultivations in medium 3.
* data provided by external partners

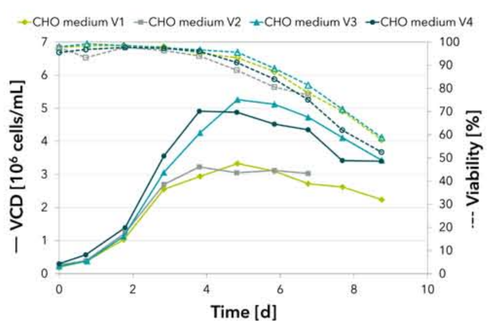
RESULTS - CHO cell lines

1. Transfection



Transfection efficiencies measured in two early development stages of CHO medium A and B 48 h post transfection (n ≥ 4 independent experiments). Additionally, for two CHO cell lines, the expression of a monoclonal antibody was measured 48 h post transfection. The cultivation before and after transfection was performed in reference growth medium.

2. Cell growth



For the development of a bifunctional CHO medium, four variants of medium A and B were further tested and optimized with regard to cell growth. A batch curve of transient CHO cell line 3 in these early stage variants of CHO medium B is shown.

METHODS

Cultivation All cell lines were cultivated in suspension using TubeSpin® bioreactors or shake flasks at standard conditions in either Xell medium or relevant reference media.

Transfection The setup for transfection varied for several approaches. Cells were transfected at cell densities of 3.0 and $5.0 \cdot 10^6$ cells/mL in Xell medium with a polyethylenimine (PEI) to DNA ratio of 2:1 (w/w). 0.6 to 1 pg DNA/cell were used in transfections with plasmids harbouring either a GFP gene or genes for the expression of monoclonal antibodies. Culture volumes during transfection ranged from 4 to 25 mL. 1 or 4 h after transfection, fresh medium was added to achieve a 1:2 split.

Analytics Viable cell density (VCD) and cell viability were measured using a Cedex automated cell counter. The percentage of GFP-expressing cells was determined by flow cytometry 48 h post transfection. Antibody concentration was measured by HPLC with a protein A affinity column.

CONCLUSIONS

- The developed HEK medium supports high cell densities and viability in batch cultivation.
- At the same time, transfection efficiencies ranging from 66 % to 96 % were be achieved in fresh medium.
- Considerable amounts of spent medium during transfection are still challenging. Apparently, this effect is also cell line-specific.
- Further development as well as optimization of protocols is necessary for the application of large scale transient transfections.
- Transient expression of a monoclonal antibody was successfully demonstrated. Obtained results were equal or better in the developed HEK medium compared to a reference.
- For HEK cells, titers of more than 500 mg/L were achieved after 7 days of cultivation.
- Current development stages of bifunctional CHO media already support transfection efficiencies of about 60 - 80 % while sufficient growth still requires supplementation. Work towards a one-stop medium is therefore ongoing.

PARTNERS

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