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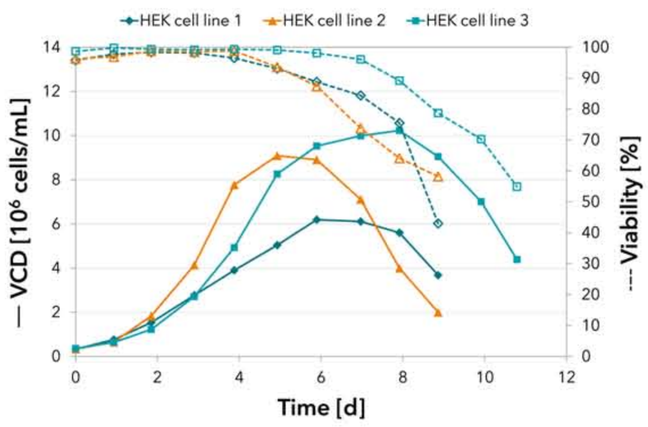
Towards a single chemically defined medium for combining transfection and cultivation of mammalian cell lines

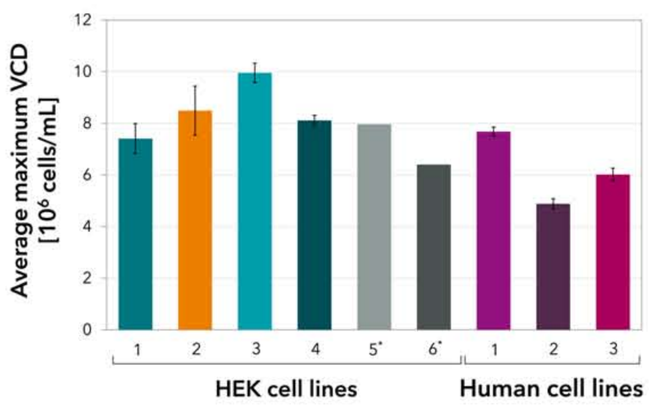
In this work, the current progress in the development of a one-stop medium for cultivation and transfection is presented. The significance of transient gene expression in mammalian cells is continuously growing. Applications range from purposes in R&D to the production of preclinical material and is often realized by a chemical method using polycationic reagents. 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

1. HEK cell growth in developed medium



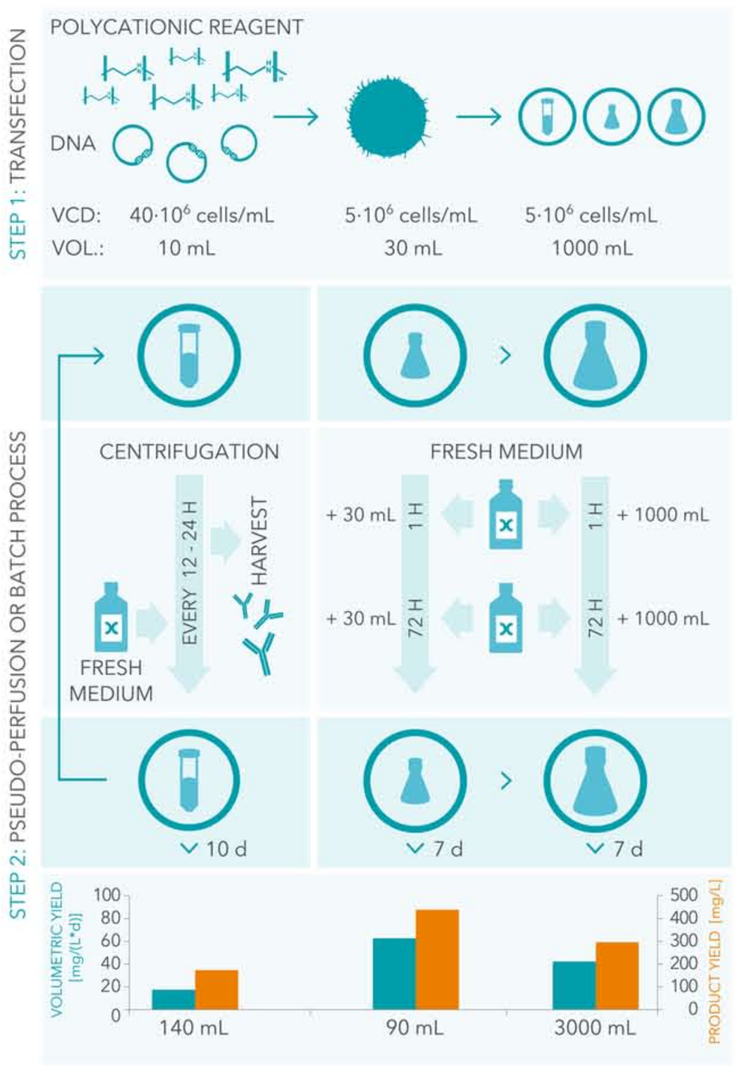
During the medium design, growth performance in different developmental stages of HEK transfection medium was evaluated for several cell lines. Cell growth was confirmed to be stable for more than 70 days during routine passaging and was also analyzed in batch shaking flask cultivations. A late step in development supports growth for more than eight days and viable cell densities (VCDs) up to $1 \cdot 10^7$ cells/mL.

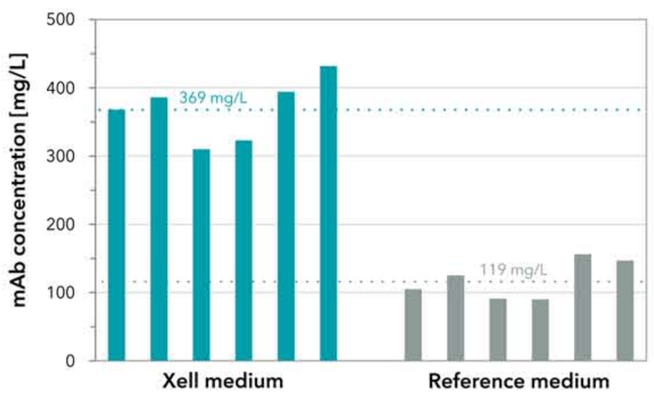


For a broad variety of six HEK and three additional human cell lines, the maximum viable cell density was determined for batch shaker cultivations in a late developmental stage. Although mainly developed with HEK cell lines, the current medium also supports cell growth for divergent human cells. (* data provided by external partners)

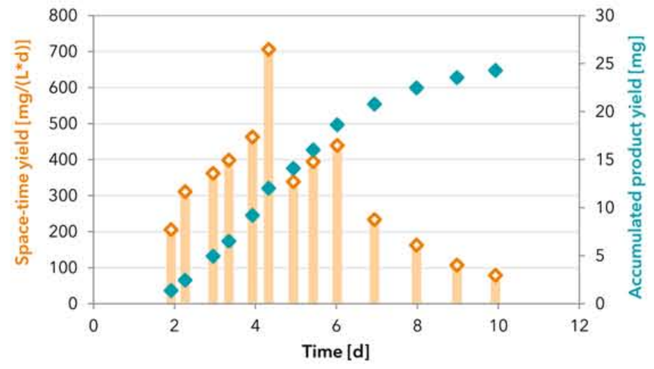
2. Process strategies and transient product yield

Transient expression of a monoclonal antibody was evaluated in several independent experiments with differing setups and scales using HEK cell line 3 and the developed HEK transfection medium. A pseudo-perfusion was achieved by centrifugation of bioreactor tubes and complete medium exchange every 12-24 hours. In batch experiments, fresh Xell medium was added twice after transfection.



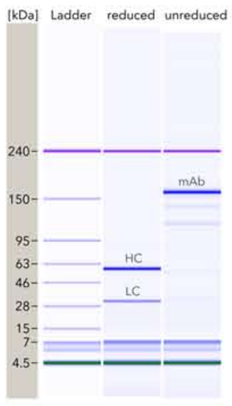


The yields of transient monoclonal antibody expression with HEK cell line 3 were measured in several independent experiments utilizing the developed transfection medium. Titers were determined seven days post transfection and compared to results obtained with a commercially available reference medium suitable for growth and transfection (mean values are depicted by dotted lines).



For the 10 mL pseudo-perfusion process, harvest was taken every 12-24 h and replaced by fresh medium. The space-time yield at each harvesting step increased until day four when a maximum value of 706 mg/(L·d) was reached. Accumulated titers added up to a final yield of 24.3 mg after ten days of the process.

Harvest from pseudo-perfusion and batch processes was subjected to dialysis and analyzed by capillary SDS-PAGE to evaluate protein purity as well as integrity. The figure shows exemplary results for the 90 mL batch process. Bands at 240 kDa and 4.5 kDa result from upper and lower marker proteins included in the analyses for alignment. Under reduced conditions, only bands for the heavy (HC) and light chain (LC) are visible. For unreduced conditions, slight impurities are detectable in non-purified harvest.



CONCLUSIONS

- The developed HEK transfection medium supports stable cell growth in seed train as well as high cell densities and viability in batch cultivation.
- Transfection efficiencies between 66 to 96 %, depending on cell line, were achieved in fresh medium of the current developmental stage.
- Considerable amounts of spent medium during transfection are still challenging. The degree of this effect is also cell line specific.
- Targeted development as well as optimization of protocols have already enabled successful transient transfections in liter scale.
- Protein yields with current stage of medium development were factor three higher compared to a commercially available reference.
- For HEK cells, titers of up to 440 mg/L were achieved after seven days of cultivation.
- The successful pseudo-perfusion shows the feasibility of this process strategy and is simply scaled-up to bioreactor scale. Such perfusion processes for transient production are highly interesting for labile products (e.g. transient virus production or sensitive enzymes).

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. In general, cells were transfected at cell densities of $5 \cdot 10^6$ to $40 \cdot 10^6$ cells/mL in Xell medium with a polycationic transfection reagent. 2 pg DNA/cell were used in transfections with plasmids harbouring genes for the expression of a monoclonal antibody. Culture volumes during transfection ranged from 10 to 1000 mL. In batch processes, fresh medium was added twice (1 and 72 hours post transfection). A pseudo-perfusion was performed in tube biorectors with 10 mL starting volume. Medium was exchanged every 12-24 hours during the process.

Analytics Viable cell density (VCD) and cell viability were measured using a Cedex automated cell counter. Antibody concentration was measured by HPLC with a protein A affinity column. Purity evaluation via capillary SDS-PAGE was performed using an Agilent 2100 Bioanalyzer with respective protein-80 or protein-230 analysis chips.

PERSPECTIVES

- Currently, the focus in the development for HEK cells is on specialized feeds and feeding strategies to slow down proliferation while at the same time continue high recombinant protein expression in prolonged fed-batch processes.
- For CHO cells, work towards a real one-stop medium continues. Current development stages already support transfection efficiencies of about 60 - 80 % while sufficient growth still requires supplementation.

PARTNERS & ACKNOWLEDGEMENTS

Parts of this work were financially supported by the Federal Ministry of Education and Research - BMBF. Responsibility for the content lies with the author.