Püngel S.[1], Welsink T.[1], Villegas Soto P.[1], Weglöhner W.[1], Beckmann T.F.[2], Eickmeier I.[2], Northoff S.[2], Heinrich C.[2]

1 InVivo Biotech Services, Henningsdorf, Germany
2 TeutoCell AG, Bielefeld, Germany

Development of a chemically defined cultivation and transfection medium for HEK cell lines

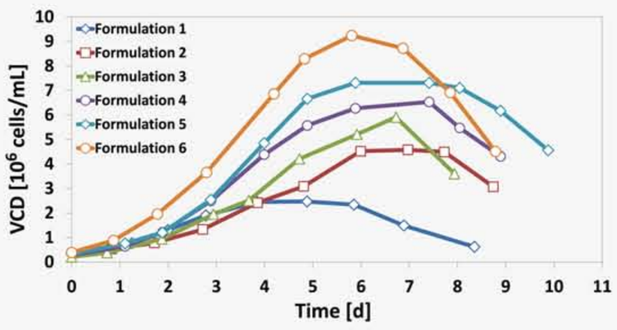
In this work, the current status in the development of media supporting cell growth, transfection and production in HEK cells is presented. Finally, processes will no longer be limited by media exchange prior transient transfection.

In the process of generating a production cell, introduction of the gene of interest into the host cell can be performed by various physical, chemical or biological methods. Because of their major advantages, chemical methods are of great interest. However, up to now upscaling is limited by the challenge to transfect cells in conditioned media with the widely used reagent PEI. Our results give a first insight into the underlying causes and first development steps for a combined growth and transfection medium are presented.

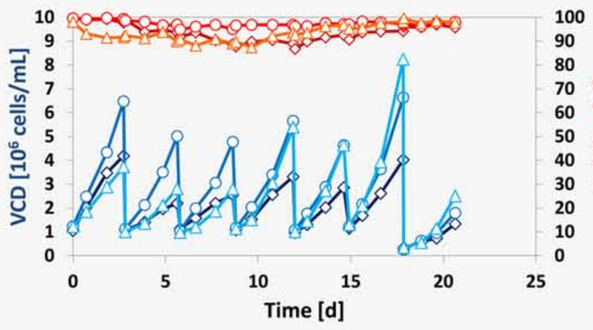
RESULTS

Growth characteristics in developed media

Batch growth for an exemplary HEK host cell line in different formulations during ongoing development of the basic growth medium carried out under uncontrolled conditions in shake flasks. Maximum viable cell density reached in formulation 6 ranged from 0.4 to nearly 1 x 10^7 cells/mL, depending on the cell line used.

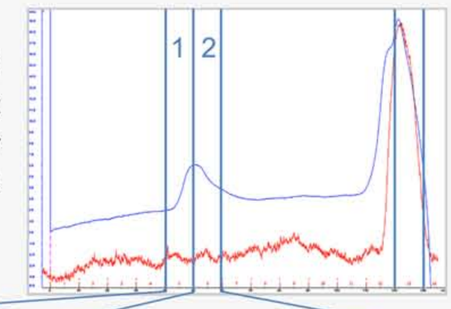


Direct adaption of three different adherent serum-depending host cell lines was successful in the latest formulation of the basic growth medium. Easy protocol for adaption to suspension growth is of special interest during growth medium development.



MALDI-TOF/TOF analysis of conditioned media fractions

Conditioned media was subjected to SEC, high molecular weight fractions 1 & 2 were used for transfection experiments and analyzed by MALDI-TOF/TOF-MS.



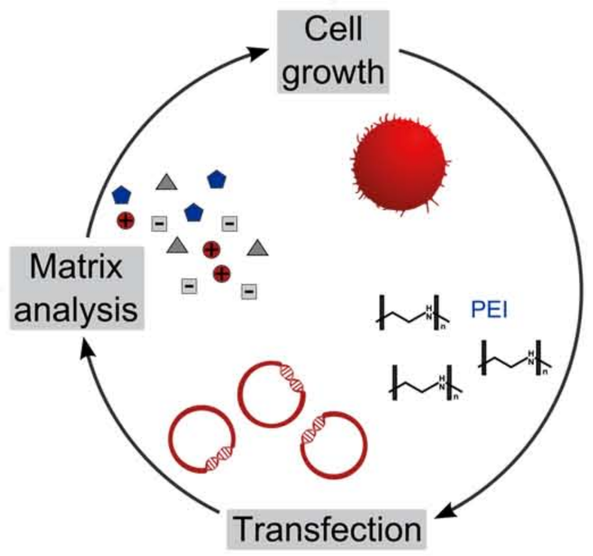
high molecular weight fraction 1

Group	Protein name	# peptides
Histones	Histone H2A	3
	Histone H2B	2
	Histone H4	2
Cytoskeleton	Tubulin alpha	2
	Tubulin beta	2
	Actin	3
Other	Galectin-3-binding protein	6
	Heat shock 70 kDa protein 1A/1B	5

high molecular weight fraction 2

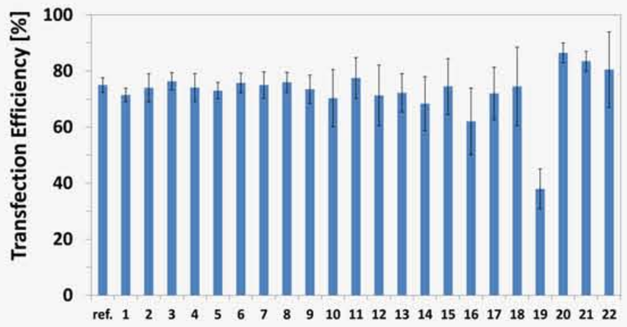
Group	Protein name	# peptides
Histones	Histone H2A	4
	Histone H2B	3
	Histone H4	4
	Hitone H3	3
Cytoskeleton	Tubulin alpha	2
	Tubulin beta	3
	Actin	6
Extracellular (matrix)	Fibrillin-2	2
	Fibronectin	5
	Clusterin	3
	Cochlin	2
Other	Galectin-3-binding protein	10
	Heat shock 70 kDa protein 1A/1B	13
	Golgi membrane protein 1	6
	Alpha-enolase	2

(identified with at least 2 peptides in up to 5 biological replicates)

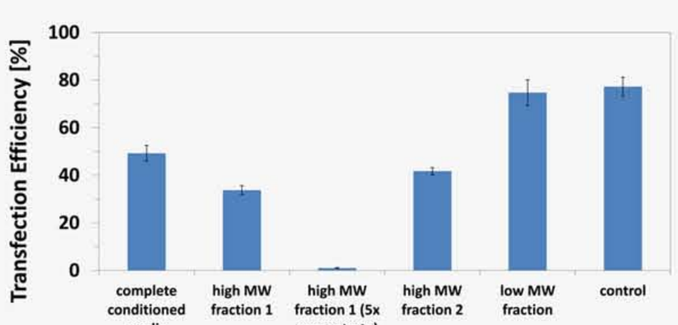


Impact of medium components and conditioned media on transfection efficiency

The screening of basal medium components exhibited no significant influence on transient transfection efficiency of HEK cells (overall efficiency of 80% ± 15%).



In contrast, depending on the level of conditioning, the presence of proteins in the high molecular weight fraction of these media reduced transfection efficiency up to 100%.



METHODS

Transfection

Transfection was performed according to standard protocols described in the literature. Briefly, 5 x 10^6 cells/mL were transfected with 2 pg DNA/cell and 25 kDa PEI in 4 mL transfection volume. Transfection efficiency was determined 24 hours post transfection by counting green fluorescent positive cells using a FACSCalibur (BD Biosciences).

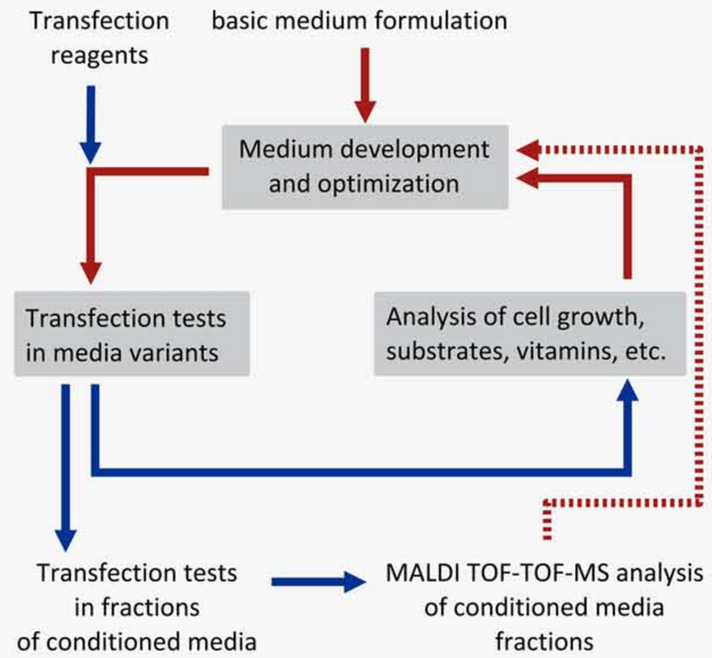
Cultivation

Cultivations of various HEK cell lines were carried out using shake flasks with commonly used standard conditions.

Analytics

Automated viable cell counting was performed by a Cedex. Furthermore, the quantities of components like glucose, lactate, amino acids, salts and vitamins in the supernatant were measured. Based on this information, single ingredients or groups of components from the basal formulation were screened for their influence on transfection efficiency. To evaluate the effect of cellular proteins in conditioned medium they were separated by size exclusion chromatography and analyzed via MALDI-TOF/TOF mass spectrometry (ultrafleXtreme, Bruker). SEC was performed using the high resolution gel filtration medium Superdex™ 200 16/60 with the ÄKTAprime system.

Workflow of transfection media development



CONCLUSIONS

- High transfection efficiencies of up to 80 % were achieved 24 h post transfection
- High molecular weight fractions of conditioned media reduce transfection efficiency, other fractions have no negative effect on transfection efficiency
- Noticably, high molecular weight fractions contained histones which might be a potential factor with negative impact on transfection efficency
- Basal medium components exhibit no influence on transient transfection
- Latest medium formulation supports cell growth of various HEK cell lines and easy adaption to suspension growth
- Major challenges for combining transfection and growth medium in one formulation are to preserve single cell growth of HEK cells while avoiding commonly used anti-aggregation components

PARTNERS & CONTACT

INVIVO InVivo BioTech Services GmbH
www.invivo.de
info@invivo.de

TeutoCell Cutting-edge competence in cell culture technology
www.teutocell.de
info@teutocell.de

Supported by:
Federal Ministry of Economics and Technology
on the basis of a decision by the German Bundestag

ZIM Zentrales Innovationsprogramm Mittelstand