SERUM-FREE PRODUCTION OF GAG-BASED VIRUS-LIKE PARTICLES BY PEI-MEDIATED PLASMID TRANSIENT TRANSFECTION IN MAMMALIAN SUSPENSION CELLS

Part I: Optimization of HEK 293 cell growth by addition of non-animal derived components using design of experiments

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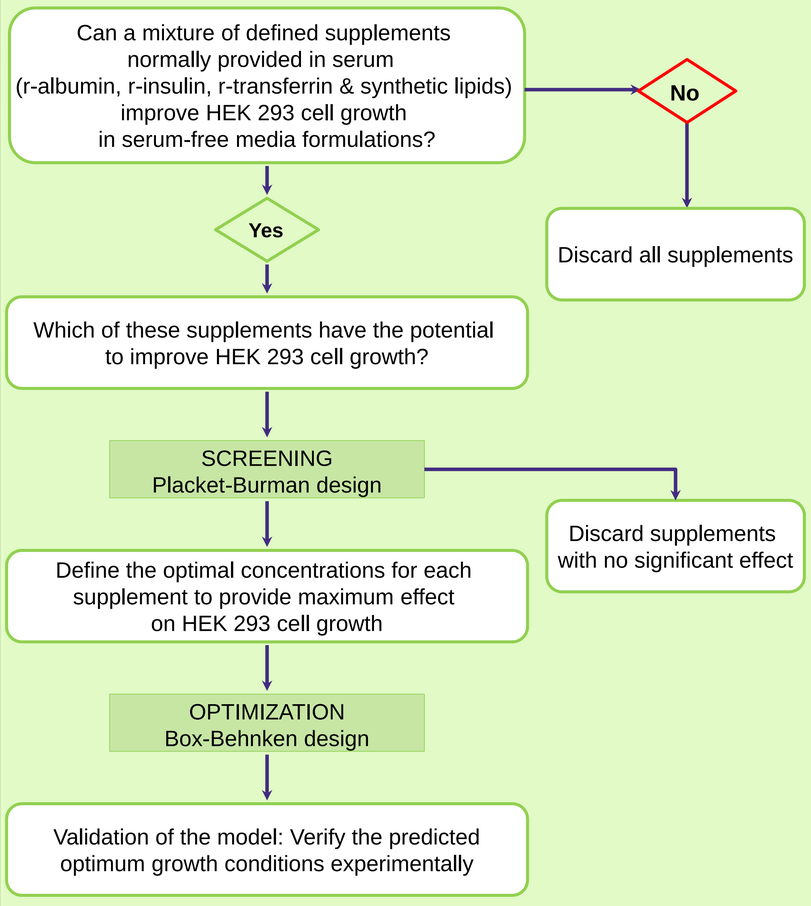
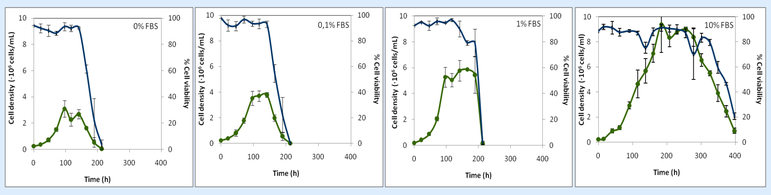
ABSTRACT

Mammalian cells are a widely used expression platform for the production of recombinant therapeutic proteins or viral particle-based vaccines since they typically perform appropriate protein post-translational modifications and authentic viral particle assembly. Of the available mammalian cells, HEK 293 is one of the most industrially relevant cell lines because it is cGMP compliant and is able to grow in suspension in a variety of serum-free media. Of note, production of human therapeutics in mammalian cell culture has become more and more stringent in past recent years and not only demands serum-free but also animal-component free production conditions to ensure safety. The aim of this project was to optimize HEK 293 cell growth by addition of non-animal derived components to serum-free and protein-free media through design of experiments (DoE) in order to maximize productivity of a recombinant VLP vaccine by PEI-mediated transient transfection. We have analyzed the kinetics of HEK 293 cell growth in Medium 1. The cells grow to a maximum concentration of ~ 3×10^6 cells/ml with over 90% viability and show an average doubling time of 24 h. In addition, we have evaluated cell growth in two other commercial serum-free culture media (Medium 2 and Medium 3), that are also compatible with PEI-mediated transient transfection, observing similar cell growth compared to those obtained in Medium 1. We have evaluated the effect of foetal bovine sera (FBS) in these serum-free culture media. Cells can triplicate their maximum cell densities ($9,3 \times 10^6$ cells/ml) in the presence of 10% FBS. Due to the important effect of serum on HEK 293 cell growth, we decided to evaluate the effect of non-animal derived serum components on cell growth in attempt to improve cell densities while keeping animal-free production conditions. For these studies, we have selected 3 recombinant proteins (albumin, transferrin and insulin) and an in-house lipid mix composed of synthetic cholesterol, fatty acids, tocopherol and emulsifying agents. The optimal combination of these components in the final formulation has been determined by using DoE. Results have shown that by adding a mixture of animal-free supplements normally provided in serum to serum-free cell culture medium, it is possible to reach cell densities comparable to those attained in the presence of 10% FBS while avoiding the problems derived from its use.

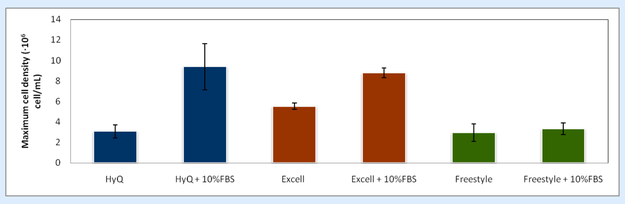
EXPERIMENTAL APPROACH



UNKNOWN SERUM COMPONENTS HAVE THE POTENTIAL TO IMPROVE HEK 293 CELL GROWTH IN SERUM-FREE MEDIA FORMULATIONS

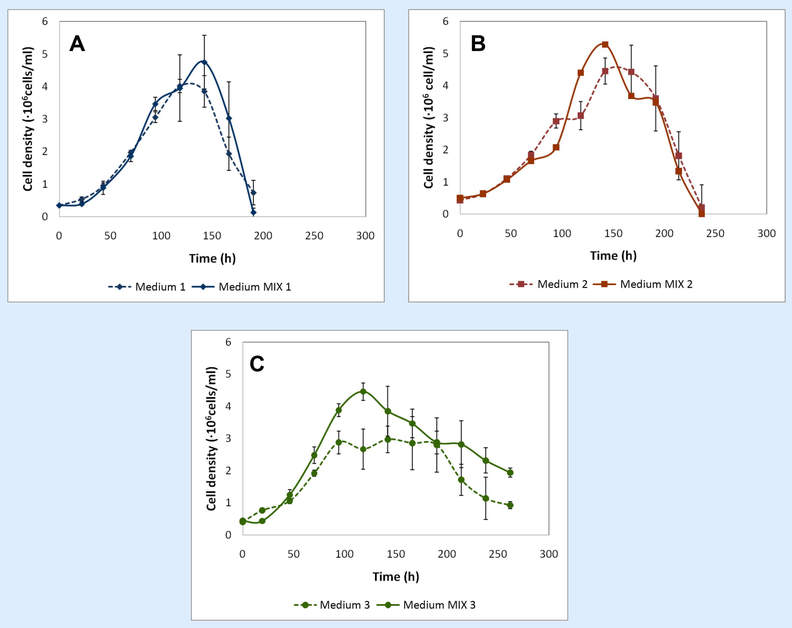


The effect of different concentrations of FBS on HEK 293 cell growth
HEK 293 cell growth kinetics in Medium 1 serum-free culture medium supplemented with various concentrations of FBS. Cell density (green line) and viability (blue line) are shown. Values presented are the mean ± SD (n=3).



The effect of FBS supplementation in commercially available serum-free media
Maximum viable cell density reached by HEK 293 cells in Medium 1, Medium 2 and Medium 3 serum-free culture media in the presence or absence of 10% FBS. Values presented are the mean ± SD (n=3).

EFFECT OF A PREDEFINED MIXTURE OF SUPPLEMENTS ON HEK 293 CELL GROWTH



Growth kinetics of HEK 293 cells in 3 different serum-free formulations in the presence or absence of a pre-defined mixture of supplements
HEK 293 growth curves in Medium 1 (blue), Medium 2 (red) or Medium 3 (green) serum-free formulations in the presence (solid lines) or absence (dashed lines) of supplementation with r-Albumin (1 g/L), r-Insulin (10 mg/L), r-Transferrin (10 mg/L), and an in-house developed lipid mix (1X) at concentrations recommended in the literature (Ref. 1). Values presented are the mean ± SD of triplicate experiments. HEK 293 cell density is improved in the presence of the mix, but only in Freestyle medium a significant difference is observed. This medium was selected for further optimization by DoE.

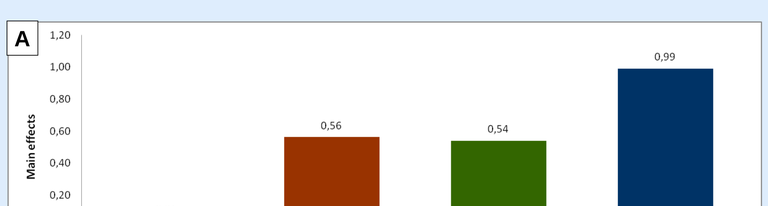
SCREENING SUPPLEMENTS WITH SIGNIFICANT EFFECT ON HEK 293 CELL GROWTH

Independent variables	Code levels	
	Low (-)	High (+)
r-Albumin (g/L)	0	1
r-Insulin (mg/L)	0	10
r-Transferrin (mg/L)	0	10
Lipid Mix (X)	0	1

Placket-Burman design
Screening of supplements with significant effect on HEK 293 cell growth using Placket-Burman design (Ref. 2). The table shows the assigned concentrations of variables at two levels: low (no additive) and high (literature recommended).

EXP	Albumin	Insulin	Transferrin	Lipid Mix	Max. cell density (a)	Max. cell density (b)
1	+	+	+	+	4,8	5,0
2	−	+	−	+	4,8	4,2
3	−	−	+	−	3,7	3,8
4	+	−	−	+	3,8	3,7
5	−	+	−	−	4,0	3,2
6	−	−	+	−	2,8	3,0
7	−	−	−	+	4,0	4,3
8	+	−	−	−	3,0	2,9
9	+	+	−	−	3,2	3,1
10	+	+	+	−	4,4	4,4
11	−	+	+	+	4,5	5,2
12	+	−	+	+	4,6	4,4

Placket-Burman results
Cell density values presented are in million cells/mL



B	Coefficient	SE	t	P
Constant	3,877	0,123	31,56	<0,001
r-Albumin	-0,0227	0,123	-0,185	0,859
r-Insulin	0,297	0,123	2,417	0,046
r-Transferrin	0,311	0,123	2,532	0,039
Lipid Mix	0,573	0,123	4,666	0,002

C $Yi = \beta o + \Sigma\, \beta i\, Xi$

$Yi = 3,877 - 0,0227 \times r\text{-Alb} + 0,297 \times r\text{-Ins} + 0,311 \times r\text{-Trans} + 0,573 \times LipMix$

Effect of recombinant proteins and synthetic lipids on HEK 293 cell growth
Using a Placket-Burman design of experiments we were able to determine in 12 experimental runs (performed in duplicate) that r-Insulin, r-Transferrin and an in-house developed lipid mix positively affect HEK 293 cell growth in serum-free media formulations, whereas r-Albumin showed no significant effect (A, B & C).

DEFINING OPTIMAL SUPPLEMENT LEVELS FOR MAXIMUM HEK 293 CELL GROWTH

Independent variables	Code levels		
	-1	0	1
r-Insulin (mg/L)	1	10	20
r-Transferrin (mg/L)	1	10	20
Lipid Mix (X)	0,1	1	2

Box-Behnken design
Optimal concentrations for each supplement showing a significant effect on HEK 293 cell growth were defined based on a Box-Behnken experimental design (Ref. 3). Three levels of concentrations for each variable including a maximum (1), a minimum (-1) and a center point (0) were selected based on the literature.

EXP Nº	Insulin	Transferrin	Lipid Mix	Max. cell density	Predicted cell density
1	-1	-1	0	3,1	2,8
2	1	-1	0	5,5	5,4
3	-1	1	0	3,7	3,8
4	1	1	0	3,3	3,7
5	-1	0	-1	3,1	3,1
6	1	0	-1	4,6	4,4
7	-1	0	1	2,0	2,3
8	1	0	1	3,6	3,6
9	0	-1	-1	3,2	3,6
10	0	1	-1	3,5	3,4
11	0	-1	1	2,8	2,9
12	0	1	1	2,8	2,4
13	0	0	0	4,9	4,5
13	0	0	0	4,9	4,5
13	0	0	0	3,8	4,5

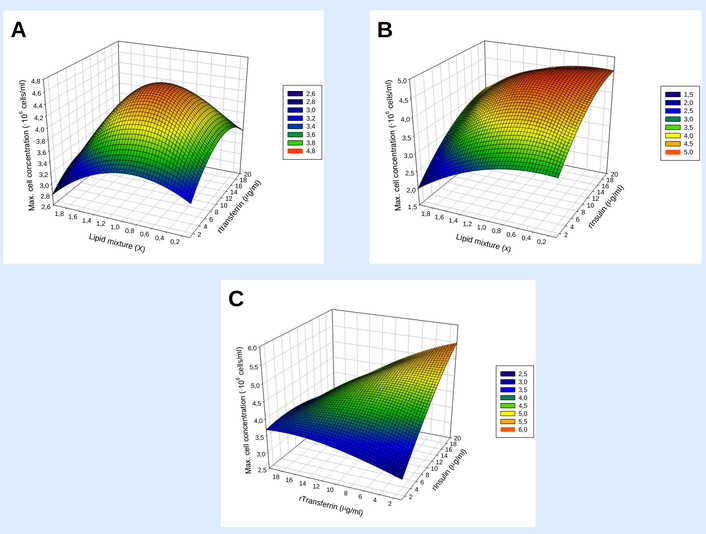
Box-Behnken results
Cell density values presented are mean of duplicate runs in million cells/mL

A $Yi = \beta o + \Sigma\, \beta i\, Xi + \Sigma\, \beta ii\, Xi^2 + \Sigma\, \beta ij\, XiXj$

$Yi = 4,5333 + 0,635 \times r\text{-Ins} - 0,1675 \times r\text{-Trans} - 0,4125 \times LipMix - 0,68 \times r\text{-Ins} \times r\text{-Trans} + 0,02 \times r\text{-Ins} \times LipMix - 0,075 \times rTrans \times LipMix - 0,1792 \times r\text{-Ins}^2 - 0,4542 \times r\text{-Trans}^2 - 1,0242 \times LipMix^2$

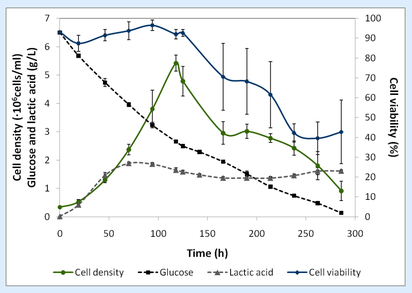
B	Optimum levels of additives
r-Insulin (mg/L)	19,8
r-Transferrin (mg/L)	1,6
Lipid mix (X)	0,9

Box-Behnken model accurately predicts HEK 293 cell densities
Using a Box-Behnken design of experiments, we were able to define in 15 experimental runs (performed in duplicate) a model (A) that accurately predicts HEK 293 cell concentrations in the presence of different concentrations of r-Insulin (r-Ins), r-Transferrin (r-Trans) and lipid mix (LipMix). Optimal concentrations for each supplement were defined (B).



Response surface graphs
HEK 293 cell growth as a function of the concentrations of Lipid Mix (X) vs. r-Transferrin (mg/L) (A), Lipid Mix (X) vs. r-Insulin (mg/L) (B) and r-Transferrin (mg/L) vs. r-Insulin (mg/L) (C) based on Box-Behnken experimental results.

VALIDATION OF THE MODEL



Growth kinetics of HEK 293 cells in optimized cell culture medium
Cell density, viability, glucose and lactic acid concentrations are shown. Values presented are the mean ± SD (n=3). The maximum cell concentration reached was $5,4 \times 10^6$ cells/mL, same value as predicted using the Box-Behnken model.

REFERENCES

1- Keenan et al., 2006. Cytotechnology, 50: 49-56
2- Plackett, R.L., Burman, J.P., 1946. Biometrika, 33: 305–325.
3- Box, G.E.P., Behnken, D.W., 1960. Technometrics, 2: 455–475.

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