A metabolic network-based approach for developing feeding strategies for CHO cells to increase monoclonal antibody production

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Abstract

Background:

Chinese hamster ovary (CHO) cells are the main workhorse in the biopharmaceutical industry for the production of recombinant proteins, such as monoclonal antibodies. To date, a variety of metabolic engineering approaches have been used to improve the productivity of CHO cells. While genetic manipulations are potentially laborious cell line development and validation in mammalian cells, rational design of CHO cell culture medium or efficient fed-batch strategies are more popular metabolic engineering approaches.

Results:

In this study, a genome-scale metabolic network model of CHO cells was used to design feeding strategies for CHO cells to improve monoclonal antibody (mAb) production. A number of metabolites, including threonine and arachidonate, were suggested by the model to be added into cell culture medium. The designed composition has been experimentally validated, and then optimized, using design of experiment methods. About a two-fold increase in the total mAb expression has been observed using this strategy.

Conclusions:

Our approach can be used in similar metabolic engineering problems, in order to suggest new ways of increasing production in different cell factories.

Keywords:

Metabolic network models; Constrain-based modeling; DoE; Feeding strategies;

Plackett-Burman; Central composite design.

Background

Bio-based production of industrially-relevant or pharmaceutically-important proteins, such as monoclonal antibodies (mAbs), has been a major goal of biotechnology in the past decades (1). MAbs have diverse diagnostic and therapeutic applications, especially for cancer and autoimmune diseases (2). The existence of certain glycosylation patterns on some mAbs restricts the choice of host cell lines for their production to mammalian cell lines, including Chinese hamster ovary (CHO) cells.

Several studies have focused on optimizing the production of recombinant proteins in CHO cells (3-5). In such studies, different omics approaches were used for understanding the reasons of higher levels of mAb production in a selected CHO cell line (6-8). Several genetic manipulation methods have been used to design super-producer CHO cells (9). However, such methods can be potentially laborious for mammalian cells. Therefore, bioprocess optimization methods, including rational design of feeding strategies or finding optimized composition for cell culture medium, are attractive for increasing productivity of CHO cells (10, 11).

Growth and productivity of CHO cells are under the direct influence of cell metabolism (12). To simulate the metabolism of cells, genome-scale metabolic network models (GEMs) can be very useful. GEMs represent *in silico* models of metabolism. A standard GEM includes organism-specific biochemical reactions together with accurate gene-protein-reaction (GPR) relations. Using reliable

metabolic models, one can perform virtual experiments in a rapid and cheap manner (13). Therefore, GEMs are considered as helpful tools in cell biology and metabolic engineering, because of their potential for predicting the metabolic state of cells under certain growth conditions.

In this study, a GEM of CHO cells, called *i*CHO1766 (14), has been used in order to predict strategies for increasing mAb production in CHO cells. Several constraint-based modeling methods have been developed to design new cell factories (15). In our study, the FVSEOF method (16) was used to suggest experimental ways of increasing the production of a mAb in CHO cells (Flux Variability Scanning based on Enforced Objective Flux or FVSEOF). Using FVSEOF method, it was predicted that CHO cell culture medium supplementation with 15 metabolites might be useful for increasing mAb production. These 15 metabolites consist of 7 amino acids (glutamine, asparagine, lysine, tryptophan, threonine, valine, and histidine), 3 vitamins (vitamin A, B1 and B6), and 5 other metabolites (thymidine, deoxy-cytidine, 3-methyl-oxobutyrate, deoxy-guanosine, and arachidonate). To the best of our knowledge, this is the first time that a GEM of CHO cells has been used to design a feeding strategy. In addition, among the predicted 15 metabolites used in the feeds, there are some metabolites that have not been reported as supplements in CHO cell culture ever before.

Experimental validation of the computational results, *i.e.*, adding each of these 15 metabolites to cell culture feeds, by changing one factor at a time could be laborious and time-consuming. Therefore, Design of Experiment (DoE) method has been used

in this study (17). Plackett-Burman (PB) design method is one of the most widely used DoE methods (18). PB method is based on the basic factorial design of experiments, which helps in measuring the effect of each variable component on an assumed response while holding the levels of the other components fixed, as well as when changing the levels of two or more components simultaneously (19). The number of experiments in a full factorial design is increased exponentially by increasing the number of components, while PB design is simple and needs very few experiments to screen the effects of components. However, the PB method neglects the interactions of components and only considers the main effects of them. In this study, a 20-run PB designed experiment was used to explore the effects of 15 metabolites on mAb production in CHO cells.

Statistical analysis of the results of PB design revealed that two metabolites, namely, arachidonate and threonine, can significantly improve mAb production in CHO cells. In order to find the most effective levels of concentrations of these two metabolites or increasing mAb production, response surface methodology (RSM) has been used. RSM is intended to explore the relationships between one or more response and several explanatory variables (20). It was introduced by George E. P. Box and K. B. Wilson in 1951. They suggested using a second-degree polynomial model to approximate the relationship between variables and responses. In contrast to the PB method, the interaction among variables can be determined by statistical techniques (21). Central composite design (CCD) is one of the response surface methods that aims to fit a model by least squares technique (22). Adequacy of the proposed model is then revealed using the diagnostic checking tests provided by

analysis of variance (ANOVA). Here, we used CCD to find the concentrations of threonine and arachidonate that can increase the mAb expression more effectively.

Results

In our study, a metabolic model of CHO cells, named *i*CHO1766, and a computational modeling method, named FVSEOF, were used to design feeding strategies to increase mAb production. The number of reactions and genes of *i*CHO1766 is relatively high, that limits the number of computational methods of strain design available to be tested on the model. Therefore, in our study, the FVSEOF method was used. The previous version of the FVSEOF method had been successfully used to suggest experimental ways of increasing a recombinant protein production in *Pichia pastoris* (23). The output of the FVSEOF method is a list of reactions that are suggested to be changed *in vitro* to increase mAb production. In our study, the exchange reactions of this list that had an increased consumption rate were selected to be tested *in vitro*. These selected reactions were related to the exchange of 15 metabolites, including 7 amino acids (glutamine, asparagine, lysine, tryptophan, threonine, valine, and histidine), 3 vitamins (vitamin A, B1 and B6), and 5 other metabolites (thymidine, deoxy-cytidine, 3-methyl-oxobutyrate, deoxy-guanosine, and arachidonate). In addition to exchange reactions, some internal metabolic reactions were found to influence mAb production. Using the proteomics data of CHO cell lines producing mAb in our lab (24), the enzyme expression levels of these metabolic reactions in high production cell line were compared to low production state. The predicted changes in the rates of these reactions in the FVSEOF list were in accordance with the enzyme expression changes (data not shown).

To start the experimental validation tests, some pre-experiments were performed to determine the initial concentrations of 15 metabolites in CHO cell culture medium

(proCHO5), using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). The initial concentrations of 7 amino acids and vitamin B1 in the CHO cell culture medium (proCHO5) were successfully determined (Supporting information, S1 file). It is assumed that the remaining 7 metabolites are not present in the cell culture medium.

To start experimental validation of the cell culture feeds using DoE method, we defined a concentration for each of these 15 metabolites in the feeds, which is obviously higher than the initial concentrations of metabolites in cell culture medium (proCHO5). The concentrations of amino acids were selected according to the available commercial feeds that were previously analyzed in our lab (25). The concentrations of vitamin B1 and B6 were selected according to some available patents, like US5316938A. The literature data regarding vitamin A and 5 other metabolites (thymidine, deoxy-cytidine, 3-methyl-oxobutyrate, deoxy-guanosine, and arachidonate) were not found. Therefore, we performed some pre-experiments to select suitable concentrations for these 6 metabolites (Supporting information, S2 file). The results of using DoE methods are as follow.

Plackett-Burman design

The metabolic modeling predicted that increasing consumption rates of 15 metabolites might enhance the mAb production *in vitro*. To validate this prediction, these 15 metabolites were added to the feed solutions of CHO cells, using a matrix that was designed by the Plackett-Burman (PB) method (Table 1). According to the

PB design matrix, a total of 20 trials were performed at various combinations of 'high' (1.00) and 'low' (-1.00) levels of concentrations of different metabolites. The low levels were the amount of metabolites in the CHO cell culture medium (proCHO5), and high values were determined according to the literature and some pre-experiments (Supporting information, S1 and S2 files).

Table 1. The composition of CHO cell culture feeds in Plackett–Burman (PB) design matrix. Twenty groups of feed solutions were designed at various combinations of 'high' (1.00) and 'low' (-1.00) levels of concentrations of 15 different metabolites. According to the table, group 19 was considered as the negative control, because it does not contain any of the 15 metabolites as supplements and therefore, only proCHO5 medium was added to the cells as feed 19. Glu: glutamine, Asp: asparagine, Lys: lysine, Trp: tryptophan, Thr: threonine, Val: valine, His: histidine, Vit B1: vitamin B1 or thiamine, Vit B6: vitamin B6 or pyridoxine, Vit A: vitamin A or retinol.

Group	Glu	Asn	Lys	Trp	Thr	Val	His	Vit B1	Vit B6	Thymidine	Deoxy-cytidine	3-methyl-oxobutyrate	Deoxy-guanosine	Vit A	Arachidonate
1	-1.00	-1.00	-1.00	-1.00	1.00	-1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00	-1.00	1.00
2	-1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00
3	-1.00	1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00
4	1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00
5	1.00	-1.00	1.00	-1.00	1.00	1.00	1.00	-1.00	1.00	1.00	-1.00	-1.00	1.00	1.00	1.00
6	-1.00	1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	-1.00
7	1.00	1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00	-1.00	-1.00	-1.00
8	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00	-1.00
9	1.00	-1.00	-1.00	-1.00	-1.00	1.00	-1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00	-1.00
10	1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	-1.00	-1.00
11	1.00	-1.00	1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00	-1.00	1.00	1.00	1.00	1.00	-1.00
12	1.00	1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00
13	-1.00	1.00	1.00	-1.00	-1.00	-1.00	1.00	-1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00	1.00
14	1.00	1.00	1.00	-1.00	1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	-1.00	-1.00	1.00

15	-1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00	1.00	1.00	-1.00	-1.00	1.00	1.00	1.00	1.00
16	1.00	1.00	-1.00	1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	-1.00	-1.00	1.00	1.00
17	1.00	-1.00	1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00
18	-1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	-1.00	1.00	1.00	-1.00	1.00	1.00
19	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00
20	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	1.00	-1.00	1.00	-1.00	1.00	-1.00	-1.00	-1.00

The number of viable and dead cells were counted on each day for all groups. Changes in cell viabilities and integral viable cell count (IVCC) have been shown in Fig 1. The IVCC of the harvesting day and the total mAb expression of each group were used as responses in PB design experiments (Table 2). According to Table 1, group 19 did not contain any of the 15 supplemented metabolites and therefore, this group was considered as the control group. The highest total mAb production belongs to group 12, with 438 µg, which shows more than a two-fold increase in mAb production compared to control (186 µg). The detailed statistical analysis of the responses are presented in supporting information, S3 file. In brief, the results showed that two metabolites, namely, arachidonate and threonine, are the most influential supplemented metabolites that showed significant improvements in mAb production. Therefore, these two metabolites were chosen for further analysis using the RSM method.

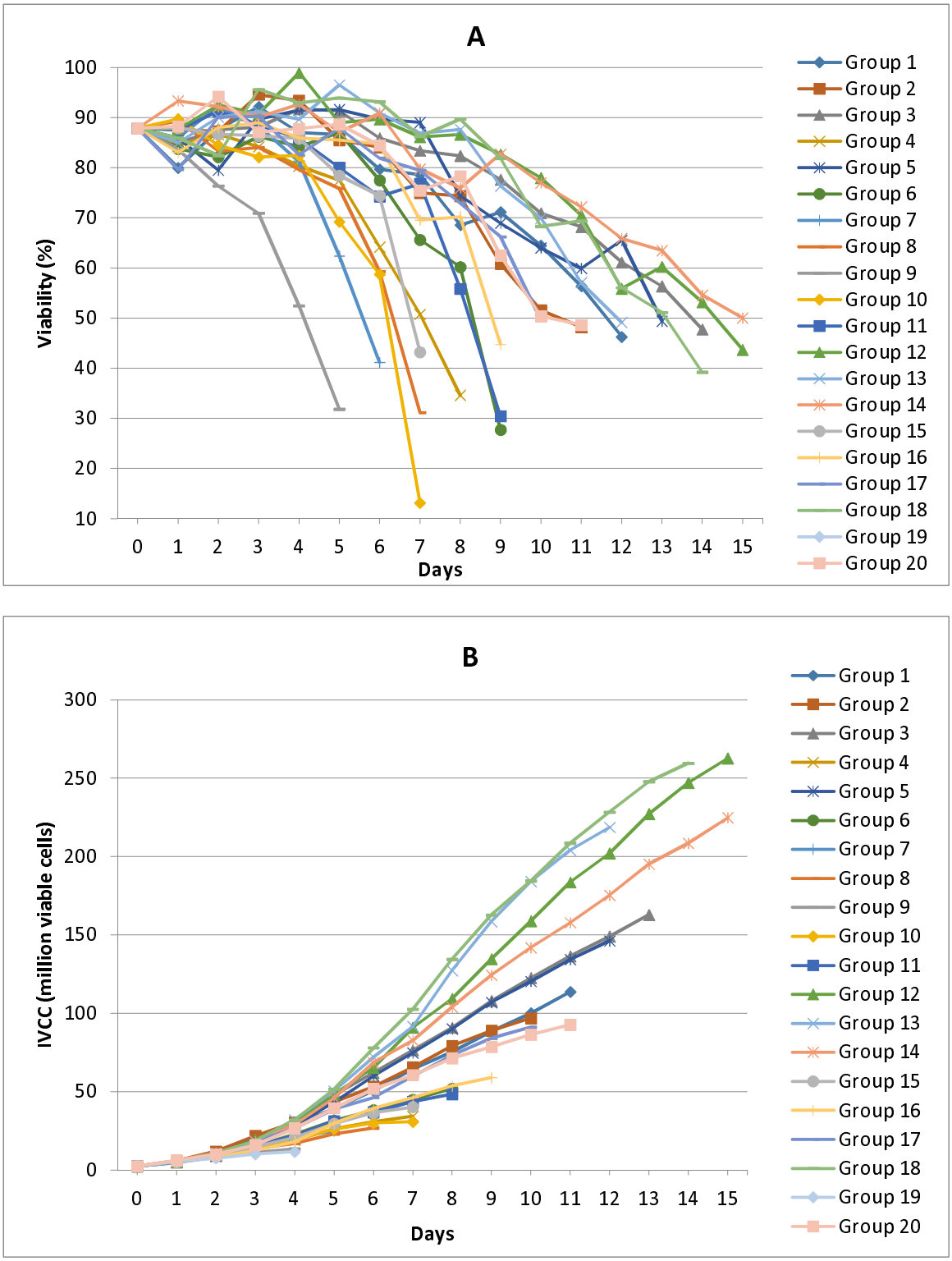

Fig 1. Changes in CHO cell viabilities (A) and integral viable cell count (IVCC) (B) of group 1-20. CHO cell viabilities have been shown by the percent of viable cells to the total number of cells. IVCC has been calculated by cumulative addition of viable cell counts in millions for each day during CHO cell

culture supplementation with 20 different feeds. The compositions of the feeds have been shown in Table 1.

Table 2. The responses of Plackett–Burman design of experiments. The first response is integral viable cell count (IVCC), which is presented in million cells for each group. The second response is total mAb production.

Group	The first response Integral viable cell count (million cells)	The second response Total mAb expression (µg)
1	122.970	328
2	103.359	297
3	173.995	419
4	37.370	224
5	156.548	375
6	55.127	222
7	27.169	189
8	28.833	178
9	15.067	173
10	30.830	175
11	50.851	218
12	262.717	438
13	218.692	375
14	224.816	415
15	40.264	172
16	58.980	219

17	91.261	229
18	259.608	434
19	11.733	186
20	92.681	309

Central composite design

Arachidonate and threonine, were the most influential supplements that could significantly improve mAb production in PB designed experiments. To find the most effective concentrations of these two metabolites, central composite design (CCD) was used. Five levels of concentrations for each of the two metabolites were tested in 11 trials in CCD (Table 3). The concentrations of metabolites in '0.00' levels in CCD were equal to '+1.00' or high levels in PB design.

Table 3. Composition of CHO cell culture feeds in CCD matrix. Eleven groups of feed solutions were designed at various combinations of '+1.00', '-1.00', '0.00', '+1.41', and '-1.41' levels of arachidonate and threonine concentrations.

Group	**Level of threonine concentration**	**Level of arachidonate concentration**
1	0.00	0.00
2	0.00	-1.41
3	-1.00	-1.00
4	1.00	-1.00

5	0.00	0.00
6	-1.00	1.00
7	0.00	1.41
8	0.00	0.00
9	1.41	0.00
10	-1.41	0.00
11	1.00	1.00

The number of viable and dead cells were counted on each day for all groups. Changes in cell viabilities and integral viable cell count (IVCC) have been shown in Fig 2.

The IVCC of the harvesting day and the total mAb expression of each group were used as responses in CCD experiments (Table 4). The 11 groups in CCD design contained different concentrations of threonine and arachidonate (Table 3). The feed solution of the control group (group 12) was only 0.5 ml of basal CHO culture medium (proCHO5) with no additional threonine and arachidonate. All other conditions for group 12 were the same as other groups. In group 12, the IVCC was 14.437 million cells, and total mAb expression was 83 μg. Therefore, about 2.5 fold increase in total mAb expression was seen in group 11 compared to control. The detailed statistical analysis of the responses are represented in supporting information, S4 file. The 3-dimensional representations of the interaction of IVCC and total mAb expression with threonine and arachidonate concentration levels have been shown in Fig 3. It has been shown that both IVCC and mAb expression are

directly related to the concentrations of threonine and arachidonate, and these two metabolites are positively interacting with each other in the studied range of our analysis.

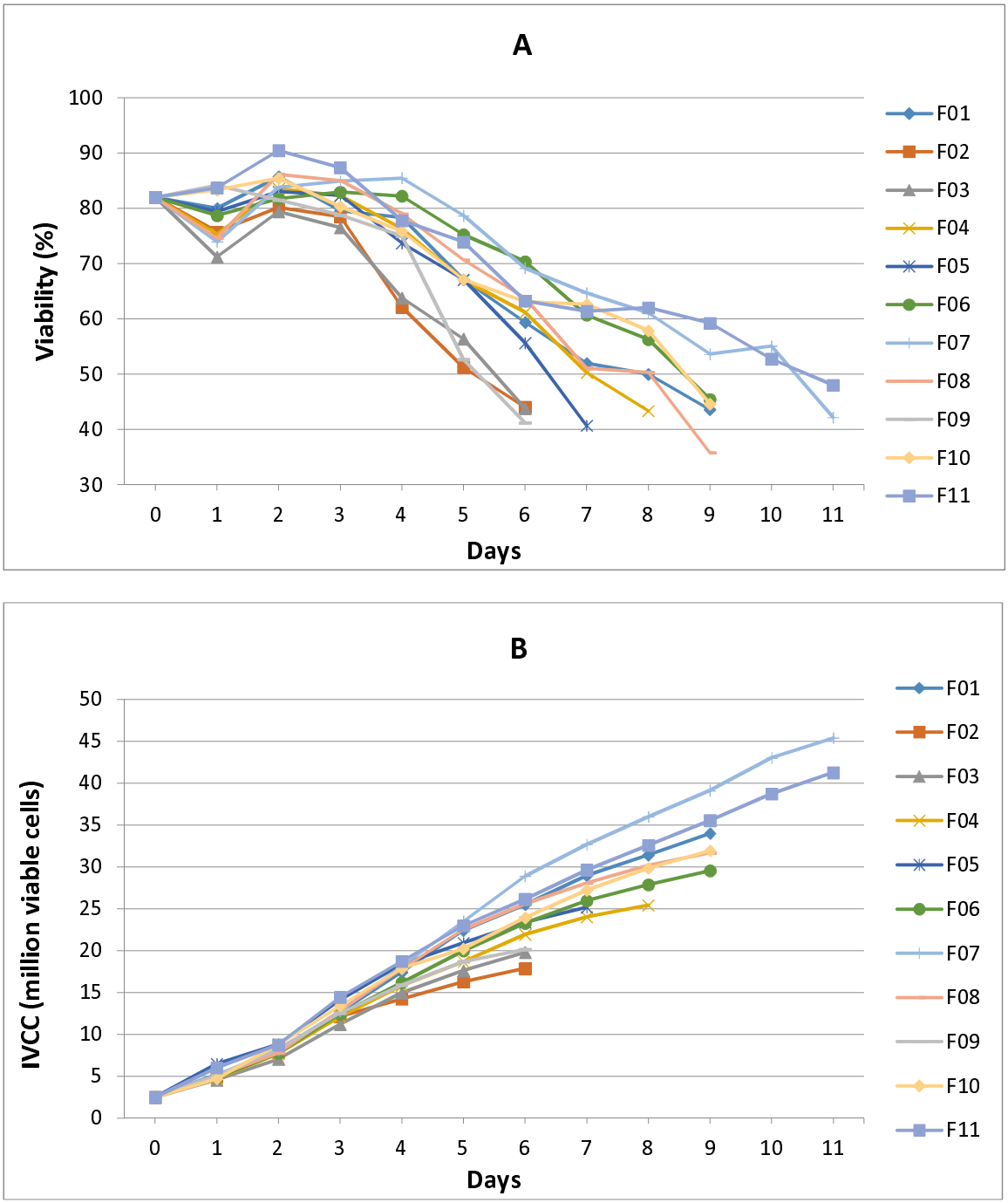

Fig 2. Changes in CHO cell viabilities (A) and integral viable cell count (IVCC) (B) during CHO cell

culture supplementation with 11 different feeds (F01-F11). CHO cell viabilities have been shown by the percent of viable cells to the total number of cells. IVCC has been calculated by cumulative addition of viable cell counts in millions for each day during CHO cell culture supplementation with 11 different feeds. The compositions of the feeds have been shown in Table 3.

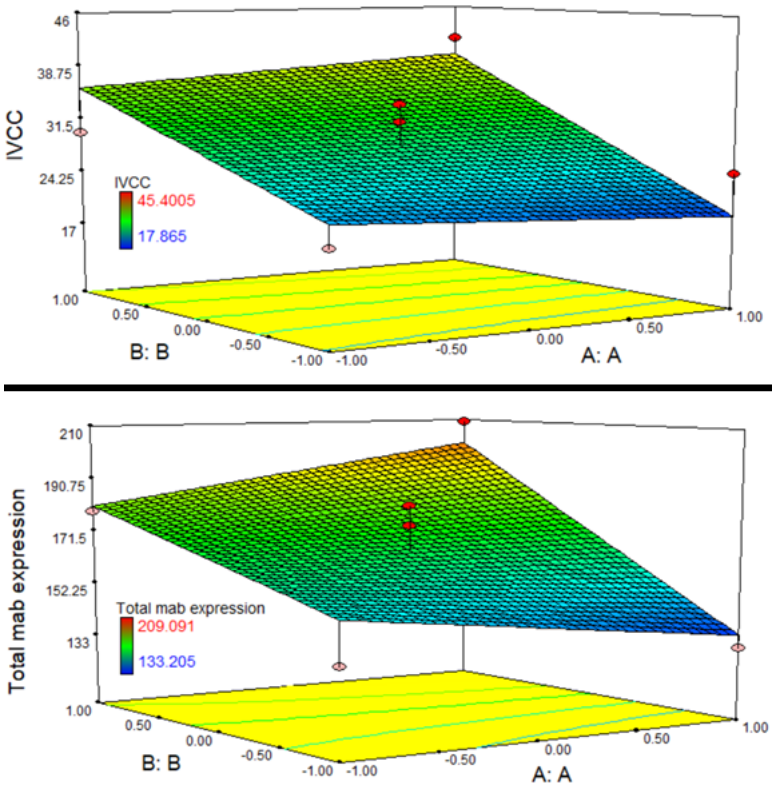

Fig 3. Representation of 3D response surface for integral viable cell count (IVCC) and total mAb expression. This response surface shows the interaction of metabolite A (threonine, on 'x' axis) and metabolite B (arachidonate, on 'y' axis) on IVCC (on 'z' axis, upper part of figure) and total mAb expression (on 'z' axis, lower part of figure). According to the statistical analysis in supporting information S4 file, IVCC = 29.29 + 0.08*A + 8.07* B + 1.52* AB and total mAb expression = 168.14 +1.12*A +22.65*B +8.84*AB. This means that both responses are directionally related to the concentrations of the two metabolites, and especially arachidonate. In addition, threonine and arachidonate are positively interacting with each other for increasing the responses.

Table 4. The responses of CCD. The integral viable cell count (IVCC) of each group is calculated in million cells. The second response is the total mAb expression, which has been determined in µg, using HPLC.

Group	The first response: Integral viable cell count (million cells)	The second response: Total mAb expression (µg)
1	34.004	182
2	17.865	140
3	19.801	138
4	25.417	133
5	25.171	156
6	29.556	179
7	45.400	186
8	31.665	175
9	20.153	170
10	31.949	181
11	41.262	209

Discussion and Conclusions

In this study, a new approach for designing the compositions of feeds for increasing mAb production in CHO cell culture have been proposed and experimentally validated. To the best of our knowledge, this is the first time that a GEM of CHO cell line has been used for cell culture medium or feeding strategy design. Using the metabolites predicted by metabolic network modeling as culture supplements in feed solutions, about a two-fold increase in total mAb expression was achieved.

According to the results, arachidonate was the most effective supplement in CHO cell culture feed for increasing mAb production. Arachidonate is one of the essential fatty acids (26). It has been shown that dietary supplementation with arachidonate can improve cellular health and growth (27). The major precursors of prostaglandins, prostacyclin, thromboxane, and eicosanoids have to be produced in the metabolism of arachidonate (28, 29). The positive effects of these arachidonate metabolites on the growth of Caco-2 cell line have been studied (30). It has been shown that arachidonate can stimulate the proliferation of fetal mouse brain cells (31), human colon carcinoma cells (32), and human breast cancer cell lines (33).

Arachidonate can be synthesized from linoleic acid (another essential fatty acid). However, some studies indicated that some of the cultured cells, including CHO cells, are unable to synthesize arachidonate from linoleate, as CHO cells lacked enzyme activity for the desaturation, which is the first and usually rate-limiting step of the desaturation-elongation sequence (34). Totally, most cells can synthesize fatty acids *in vitro*. However, *de novo* synthesis is usually relatively low and cultured cells tend to rely on exogenous free fatty acids.

We note that there are some limitations in metabolic modeling. Even in the most complete metabolic models that have been reconstructed till now, some reactions may be missing, according to a lack of knowledge in cell biology or some errors made in the process of reconstruction of the model. In addition, a metabolic model only considers the biochemical reactions occurred in a cell, while a metabolite may also have a role in transcriptional or signaling pathways. In other words, increasing the consumption of a metabolite may increase the rate of a biochemical reaction in favor of mAb production, while this metabolite may also take part in a signaling pathway and cause up-regulation of another pathway that is against mAb production or against cell viability. Integration of signaling and metabolic networks may be a solution for these limitations (35). However, there has been not any reliable signaling network for CHO cells until the writing of this study. Another limitation in metabolic modeling is choosing the right algorithm. As already mentioned, several strain design algorithms were tested in the first step of this study for analyzing the metabolic model of CHO cells, like optForce (36). However, because of the relatively big size of the metabolic model of CHO cells, and also operational limitations of computational calculations, only FVSEOF algorithm came to reliable results.

Methods

Cell culture

The CHO cell line, producing an anti-α4β7 antibody, was gifted from Radin Biotech Company, Iran. The cells were cultivated in proCHO5 cell culture medium (purchased from Lonza AG, Verviers, Belgium), supplemented with 4 mM L-glutamine, 0.1% of anti-clumping agent, 1% of pluronic F68 and 1% of Pen-Strep (Gibco, Life Technology, USA).

CHO cells were cultured in 20 ml glass bottles (Duran Schott®) with a working volume of 5 ml, incubated at 37°C with a 5% solution of CO_2, and agitated at 120 rpm. Each vessel was inoculated with a cell density of 5×10^5 viable cells/ml, when the viability was more than 80%. The cells were cultivated in fed-batch mode and were supplemented with 0.5 mL of the feed solutions on day 3, 5, 7, 9, and 11.

Feed solutions include different compositions of 15 metabolites dissolved in proCHO5 cell culture medium. Amino acid powders were purchased from HiMedia Laboratories (Mumbai, India). Vitamins and other metabolites were purchased from Sigma-Aldrich Company (Germany). All metabolites were dissolved in proCHO5 cell culture medium.

Cell viability was determined daily by Trypan Blue assay, using a Neubauer cytometer. The integral viable cell count (IVCC) was calculated by cumulative addition of viable cell count in each day of cell culture. The cells were harvested when the viabilities dropped to less than 50%. In the harvesting day, the cell culture

medium was centrifuged in 1100 rpm for 5 minutes, and the supernatant was 0.2 µm filtered and stored in -20 °C for determination of mAb concentration.

Metabolic network modeling

A genome-scale metabolic network model of CHO cells, called *i*CHO1766 (14), was used in our study. The metabolic reaction of mAb production was added to the model, according to the amino acid composition of the mAb which is produced by CHO cells *in vitro*. FVSEOF algorithm (16) was used to predict strategies for increasing the production of a mAb in CHO cells. In brief, using the FVSEOF method and the metabolic network, the effects of an enforced theoretical increase in mAb production on the rates of all other metabolic reactions of CHO cells can be modeled. Then, a list of metabolic reactions is generated as FVSEOF output. This list includes the metabolic reactions that have altered rates during the theoretical increase in mAb production. FVSEOF suggest that *in vitro* changes in the reactions of this list, according to the direction of their predicted altered rates in the list, can increase mAb production *in vitro*.

Design of Experiment (DoE) methods

The Plackett-Burman (PB) design, Central Composite Design (CCD), and statistical analysis of the results were performed using the Design-Expert software version 7 (Stat-EaseInc. Minneapolis, Minnesota, USA). Two responses were defined for both

the PB design and CCD. The first response was the integral viable cell count (IVCC) of the harvesting day. IVCC of each day was calculated by cumulative addition of viable cell counts (from the first day of cell culture until the end of each day). The second response was the total mAb expression of each group. This response was calculated by measuring the concentration of mAb in the CHO cell culture supernatant on the harvesting day, measured by HPLC (see the next section of methods).

MAb concentration determination

A MAbPac protein A affinity column (Thermo-Scientific, USA) was used for HPLC analysis. The equilibration and elution buffers have the same compositions, which includes 5% of PBS buffer and 5% acetonitrile in 0.15 M sodium chloride solution (Merck, Darmstadt, Germany). The pH of equilibration buffer was 7.2, and the pH of elution buffer was set to 2.7 using ortho-phosphoric acid. The temperature for the column was set to 30°C. The relative concentration of elution buffer to equilibration buffer was increased from 0 to 100% in approximately 3 minutes. The injection volume was 20 µL, and the flow rate was 2 ml/minute. The concentration of mAb in CHO cell culture medium was determined based on a standard curve generated with previously known concentrations of a purified monoclonal IgG as the standard solution.

List of abbreviations

CHO = Chinese hamster ovary

mAbs = monoclonal antibodies

GEMs = genome-scale metabolic network models

GPR = gene-protein-reaction

FVSEOF = flux variability scanning based on enforced objective flux

DoE = design of experiment

PB = Plackett-Burman

CCD = central composite design

Declarations

Ethics approval and consent to participate

"Not applicable"

Consent for publication

"Not applicable"

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files]. The metabolic model of CHO cells is publicly available in the supporting information of the original article (Hefzi et. al., Cell Systems, 2019), which has been cited in our article. The cell line which has been used in our study is available in Radin Biotech company of Iran.

Competing interests

"The authors declare that they have no competing interests"

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Authors' contributions

H.F. and S.-A.M. designed the computational studies. N.E.L. was involved in computational modeling of CHO cells metabolism. H.F., B.V., F.T., and F.M. designed the lab experiments. H.F. wrote the main manuscript. N.E.L., B.V., and S.-A.M. reviewed the manuscript. All authors read and approved the final manuscript.

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Supporting information

S1 file: Determining initial concentrations of the 15 metabolic in CHO cell culture basal medium (proCHO5).

S2 file: Experimental pre-tests to choose concentrations for the 15 metabolites in feed solutions

S3 file: Detailed statistical analysis of the results of Plackett-Burman designed experiments.

S4 file: Detailed statistical analysis of the results of central composite designed experiments.