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Shake it up - how efficient evaluation of media, shear protection and raw materials can enhance transfection

The yield from transient gene expression (TGE) processes depends on various parameters, e.g. transfection protocol, cell line, cultivation set-up and medium as well as feed composition.
In order to optimize such processes, extensive tests need to be performed. In this study, two HEK cell lines were evaluated with regard to medium formulation, spent medium, shaker deflection and supplementation of e.g. surfactants or trace elements (TE). Results show that HEK 293-T cells are more sensitive to varying deflections and choice of surfactants is essential. In addition, a negative impact of TE addition on GFP expression in HEK 293-F cells was observed. These TEs are known impurities of raw materials such as tyrosine and cystine. Therefore, media raw material quality needs to be considered as well.

RESULTS

Two HEK cell lines were cultivated in three different media to evaluate the impact of medium formulation and spent media on growth and TGE. In addition, it was investigated whether individual surfactants as shear stress protection or mixtures thereof can influence deflection-related variations. Finally, the impact of supplementation with components known as raw material impurities on growth and GFP expression was tested.

A. Medium and transfection protocol screening

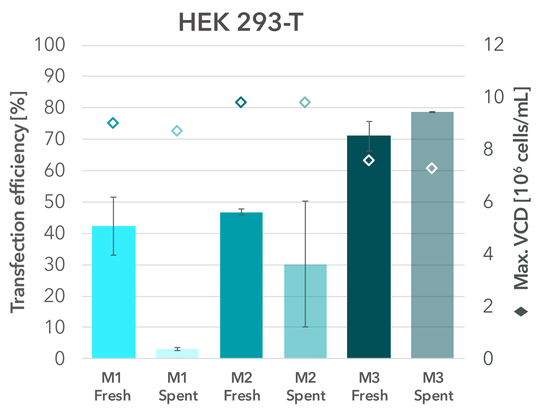

FIG. 1: Transfection efficiency (bars) and peak viable cell density (rhombs) of **HEK 293-T** cells in three different media and transfected using two different transfection protocols (transfection in fresh and spent medium). Cells were cultivated on an orbital shaker at 50 mm deflection.

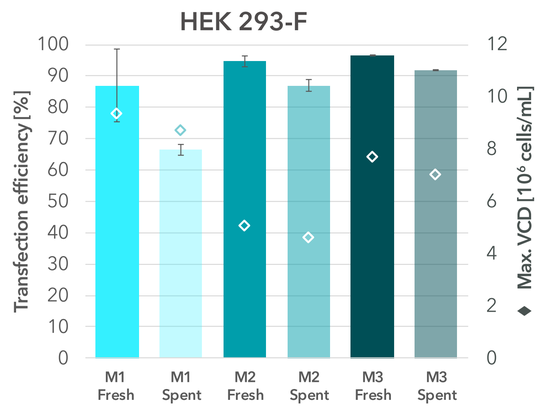

FIG. 2: Transfection efficiency (bars) and peak viable cell density (rhombs) of **HEK 293-F** cells in three different media, transfected using two different protocols. Cells were cultivated on an orbital shaker at 50 mm deflection.

B. Surfactant and deflection screening

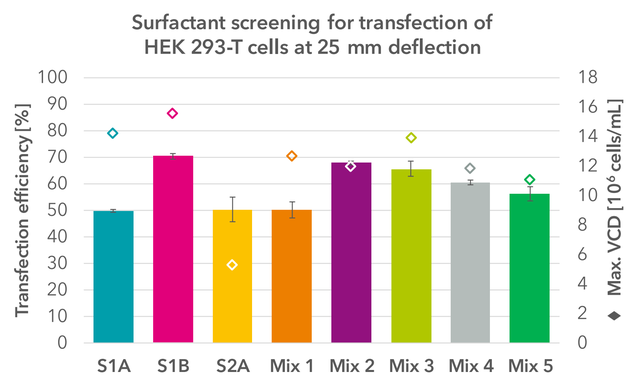

FIG. 3: Transfection efficiency (bars) and peak viable cell densities (rhombs) for surfactant screening at **25 mm deflection**, HEK 293-T cells were transfected in medium M2 with supplementation of different surfactants or mixtures thereof (Tab. 1) using spent medium transfection protocol.

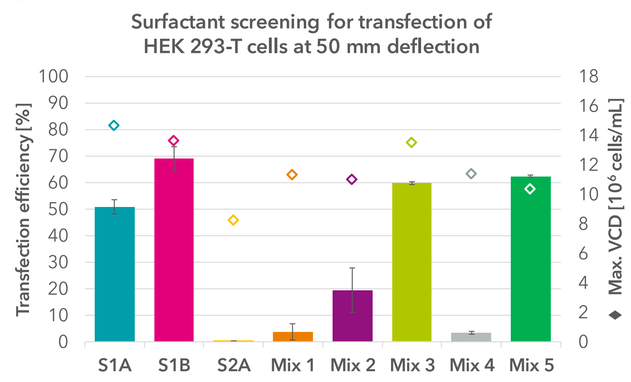

FIG. 4: Transfection efficiency (bars) and peak viable cell densities (rhombs) for surfactant screening at **50 mm deflection**, HEK 293-T cells were transfected in medium M2 with supplementation of different surfactants or mixtures thereof (Tab. 1) using spent medium transfection protocol.

C. Influence of supplements and impurities on growth & GFP expression

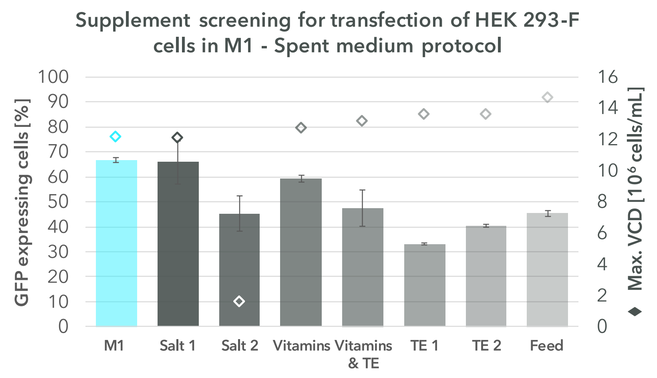

FIG. 5: Proportion of GFP-expressing cells (bars) and peak viable cell density (rhombs) of HEK 293-F cells. Supplements were tested by adding respective substances 4 h post transfection (medium M1; spent media transfection protocol) in comparable volumes to avoid dilution effects.

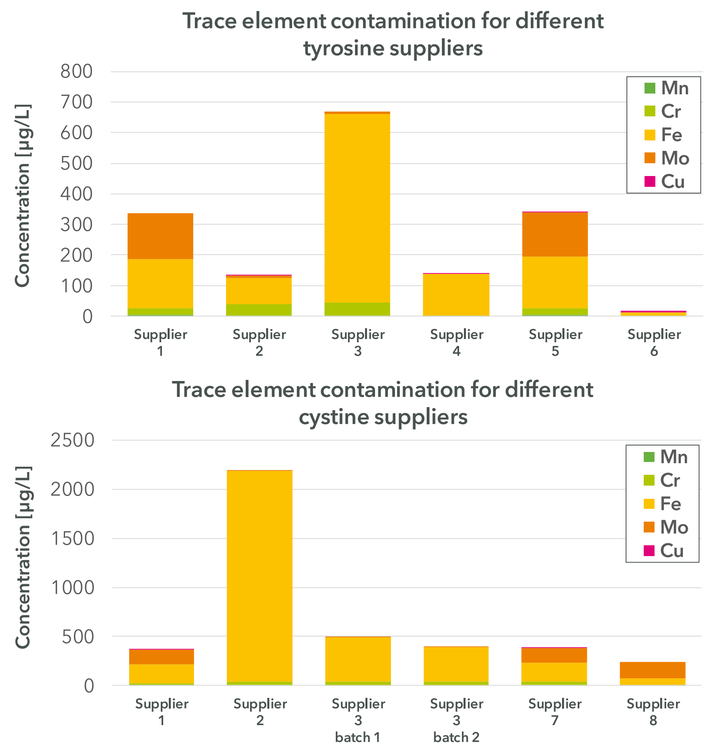

FIG. 6: Analysis of trace element impurities (Mn, Cr, Fe, Mo and Cu) in **tyrosine** batches of six and **cystine** batches of five different suppliers.

CONCLUSIONS

- To establish a transfection process with a new cell line, a media and transfection protocol screening should be performed. As shown in Fig. 1 and 2, different cell lines can show considerable deviations with regards to medium formulation and presence of spent medium.
- If insufficient transfection efficiencies are obtained, one factor to adress are type, concentration and quality of surfactants used for shear stress protection. For some cell lines this can have an important influence on cell growth and transfection efficiency.
- Differences in cell growth and TGE for the cultivation of HEK 293-T cells were observed at 25 mm and 50 mm deflection, although the energy input was kept constant.
- Negative impact of shear stress can be compensated via supplementation of either a single shear protective (Surfactant 2) or the combination of supplements (Mix 3 and 5) as shown in Fig. 4, whereas a minimum overall concentration has to be used.

Color	ID	Surfactant 1 Supplier A	Surfactant 1 Supplier B	Surfactant 2 Supplier A	Ratio	Conc.
■	S1A	x				low
■	S1B		x			low
■	S2A			x		low
■	Mix 1	x	x	x	1:1:1	low
■	Mix 2		x	x	0:1:1	medium
■	Mix 3	x	x		1:1:0	medium
■	Mix 4	x		x	1:0:1	medium
■	Mix 5	x	x	x	1:1:1	high

TAB. 1: Different surfactants from two suppliers in different qualities used in screening B (Fig. 3 and 4, as single supplementation or mixtures).

- Evaluation of different supplements such as salts, vitamins and trace elements to enhance TGE performance of HEK 293-F cells in medium M1 (Fig. 5) revealed a negative impact of trace elements on the proportion of GFP-expressing cells. Maximum viable cell densities were not effected or slightly enhanced by adding the supplements 4 h post transfection.
- A negative influence on TGE was also observed by adding a tyrosine and cystine containing feeding supplement 4 h post transfection. Both amino acids are known for their higher content of TE impurities (Fig. 6). If these impurities are one reason for reduced GFP expression needs to be further investigated.
- Based on our knowledge concerning media production, we can identify raw materials that could influence cell growth and TGE due to their impurities. Therefore, the choice of high quality raw materials is essential for the production of efficient media and feeds for TGE.

METHODS

Cultivation HEK 293-F and HEK 293-T cells were cultivated in plain shake flasks or tube spin bioreactors using standard cultivation conditions (37 °C, 80 % humidity) on an orbital shaking platform either at 25 mm or 50 mm deflection. Three different media with 8 mM glutamine, growth factor supplementation and surfactants/mixtures were tested.

Analytics Viable cell density and viability were measured daily using a Cedex automated cell counter. Transfection efficiency was quantified via GFP expression and flow cytometry 48 h post transfection. For trace element analysis, chemicals were solubilized and measured via ICP/MS.

Transfection Cells were transfected with PEI-MAX (40,000 MW) and pCMV-GFP at a ratio of 4:1. Transfections were carried out in 4 mL and 8 mL respectively in tube spin bioreactors shaken either at 25 mm or 50 mm deflection. Transfection protocols with a complete medium exchange or with 25 % spent medium were used.

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