Transient Transfection of HEK 293- EBNA Cells with Metafectene Pro, Metafectene and PEI in a Multiwell-Microbioreactor System

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Introduction

Transient gene expression (TGE) represents an attractive alternative process for rapid production of recombinant proteins, in particular, when requiring just small amounts of a few hundred milligrams as it is sufficient for structural biology or other basic research applications. In order to make TGE more competitive and effective, there is a need for optimization experiments to increase the productivity of the process while keeping costs at acceptable levels. For this purpose a multiwell-microbioreactor system was used which allows up to 48 parallel cultivations at different test conditions. Thus, a high comparability between individual process variations was achieved and combined with the potential of subsequent scale-up to shaker flasks or bioreactors. In this study we compared the efficiency of the well-known transfection reagent polyethylenimine with two transfection reagents from Biontex, namely Metafectene and Metafectene pro. Apart from investigating the transfection efficiency by means of co-expressing enhanced green fluorescent protein (eGFP) as a reporter, biolayer interferometry was used to quantify the amount of transiently produced antibodies.

Materials

A BioLector multiwell-microbioreactor (m2p Labs, Baesweiler, Germany) was used for the experiments. The quantification of antibody concentrations was measured by biolayer interferometry using an Octet®RED96 from fortéBIO (Menlo Park CA, USA). The corresponding transfection efficiency was evaluated by flow cytometry (Guava Easycyte mini, Merck Millipore, Billerica MA, USA). Metafectene Pro and Metafectene were obtained from Biontex Laboratories GmbH (Munich, Germany), and linear 25 kDa Polyethylenimin (PEI) from Polysciences (Warrington MA, USA). The plasmid pTTo/GFPq, encoding the reporter gene for investigation of the transfection efficiency, and the plasmid with the gene of interest pCMV-oriP1-scFv-hIgG1Fc (Jäger et al., 2013) were produced both in transformed *E. coli* strains using LB medium supplemented with ampicillin. Transient transfection was performed using HEK 293-6E cells (NRC-BRI, Montreal, Canada) which were cultivated in serum-free suspension in either FreeStyle F17 (Gibco, Thermo Fisher, Carlsbad CA, USA) or SMIF8 PGd 2x medium (Service-Zellkultur-Scharfenberg, Emden, Germany) (or blends thereof).

Transfection protocol

To transfect $2 \cdot 10^6$ cells mL^{-1} we used 1 mg mL^{-1} of plasmid DNA in total at a PEI/DNA ratio of 2.5:1. For Metafectene and Metafectene pro ratios of DNA to the transfection reagent were adjusted according to the recommendations of Biontex as summarized in Table I.
The different transfection reagents as well as the plasmid DNA were first diluted separately in F17 culture medium, followed by mixing and incubation at room temperature for 15 min to allow complex formation. These media containing the DNA complexes were subsequently added at a rate of 10% of the total volume to the pre-incubated HEK 293-6E cultures. Each transient transfection technology was tested in several wells in parallel using a sensor-equipped 48-round-well plate (m2p

labs) which was incubated at 37°C and 700 rpm in the BioLector. The transfection efficiency as indicated by a co-expressed eGFP reporter gene was additionally investigated by both the internal fluorescence sensor of the BioLector (online) and offline on a daily base by flow cytometry. During cultivation several additional supplements were added (e.g. tryptone TN1, SMIF8 culture medium and sodium valproate) according to our in-house protocol. The harvest of the produced recombinant antibodies was performed seven days after transfection.

Table I: Ratios of plasmid DNA to different transfection reagents

Transfection reagent	DNA conc. [mg/mL]	Conc. of transfection reagent [mg/mL]
Polyethyleneimine (PEI)	1	2.5
Metafectene pro	1	3.0
Metafectene	1	4.5

Results

As shown in Fig. 1, HEK 293-6E cells transfected using Metafectene pro resulted in a recombinant antibody production which was about 50% higher when compared to parallel cultures which were transfected using either Metafectene or linear 25 kDa PEI.

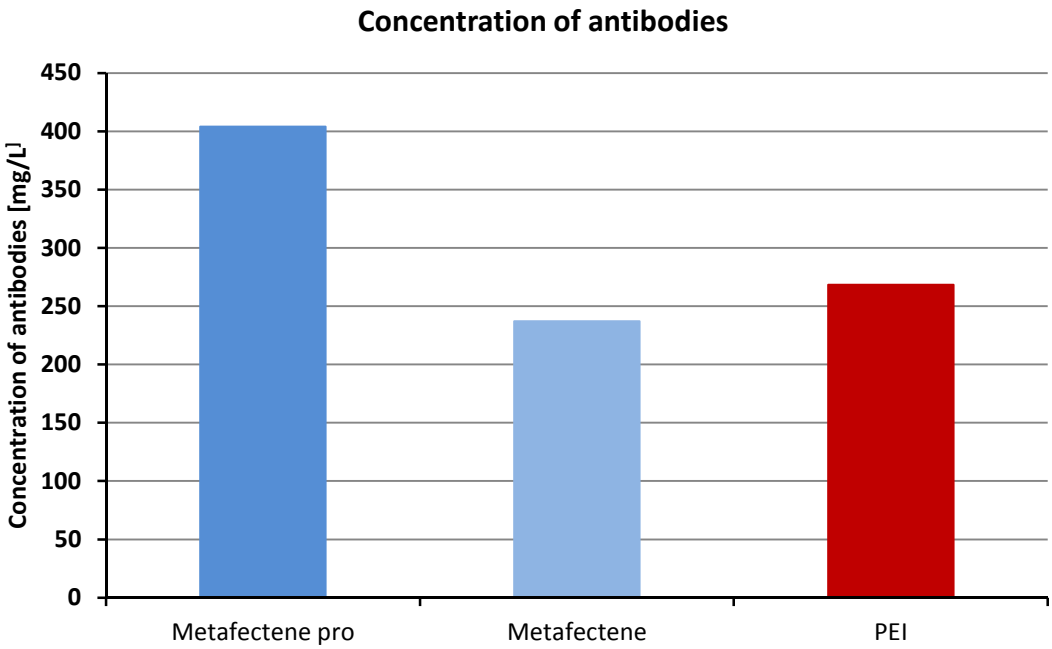

Figure 1: Accumulated antibody concentrations resulting from transiently transfected HEK 293-6E cultures using different transfection reagents as described. Each transfection method was tested in triplicate.

These data are in good correlation to the expression of the eGFP reporter gene which was analyzed both online by fluorescence monitoring in the BioLector (Fig. 2) and the calculation of the transfection efficiency after separate measurement in a flow cytometer (Fig. 3). Again, Metafectene pro was shown to be the most efficient transfection reagent.

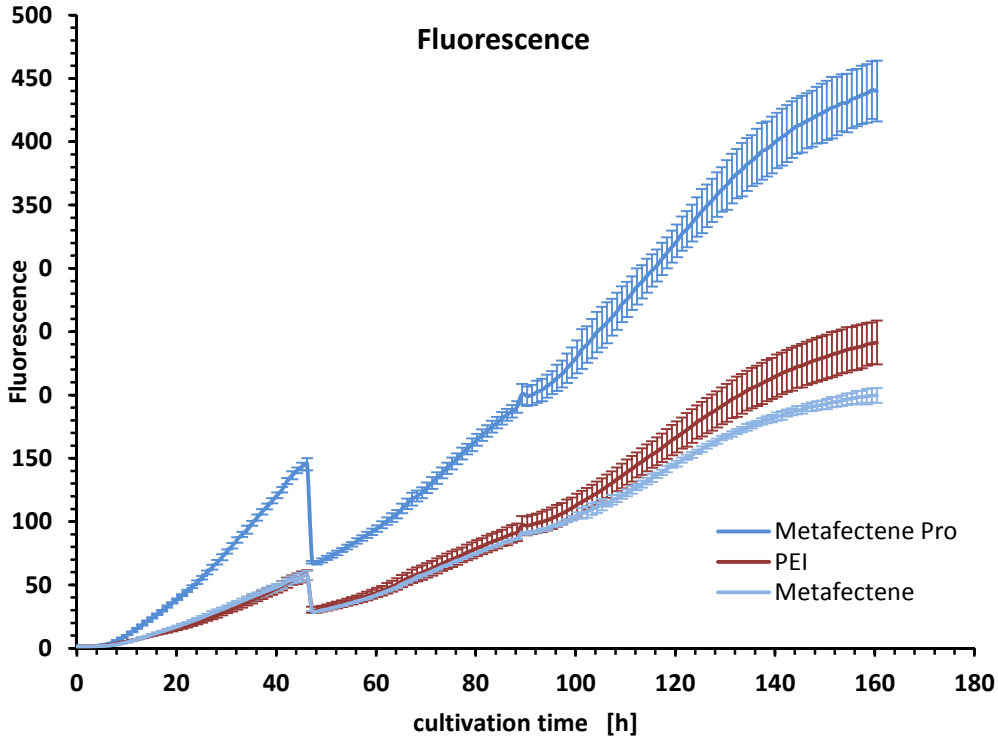

Figure 2: Online fluorescence as measured by the internal sensor of the BioLector.

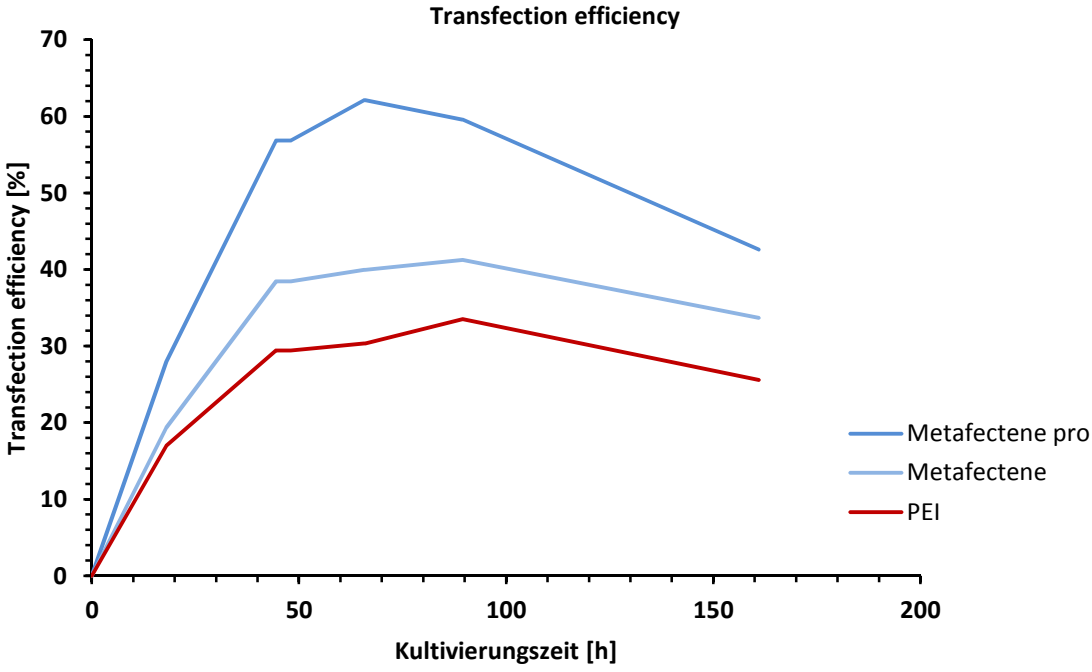

Figure 3: Ratio of fluorescent to non-fluorescent cells (and defined as transfection efficiency) as measured on a daily base by flow cytometry.

Conclusions

Metafectene pro appears to be a viable alternative to PEI for transient transfection of HEK 293-EBNA cells with regard to recombinant antibody yields. Higher costs of the transfection reagent are compensated by significantly higher product yields. The results of this preliminary study are just

based on the amount of transfection reagent and its quantitative ratio to plasmid DNA as they were recommended by the manufacturer and there appears to remain potential to further increase the yields by cell line-specific optimization of the transfection procedure with Metafectene pro.

References

Jäger, V., Büssow, K., Wagner, A., Weber, S., Hust, M., Frenzel, A., Schirrmann, T. (2013) High level transient production of recombinant antibodies and antibody fusion proteins in HEK293 cells. BMC Biotechnology **13**, 52.