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FAST AND EFFICIENT GENETIC MODIFICATION OF HUMAN CELLS UNDER SUSPENSION SERUM-FREE CONDITIONS FOR RECOMBINANT PROTEIN PRODUCTION: BY TRANSIENT TRANSFECTION OR TRANSDUCTION?

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ABSTRACT

Human cell lines have attracted great interest because they are capable of producing glycosylated proteins that are more similar to native human proteins, thereby reducing the potential for immune responses. Despite the great potential, some promising human cell lines have not been extensively exploited for recombinant protein production, especially under serum-free suspension conditions. This work aims to evaluate two strategies to genetically modify the human cell lines Huh-7, HKB-11, SK-Hep-1 to further established an efficient platform for recombinant protein production. The results obtained so far, indicate that genetic modification of the cells by lentiviral transduction rather than transient transfection seems to be the best alternative to rapidly generate high-yield recombinant cells for protein production under serum-free suspension conditions. Recombinant erythropoietin-producing cells generated by this methodology presented high expression levels.

1. INTRODUCTION

The therapeutic protein production using mammalian cell lines have become the dominant production platform, producing more than half of the biopharmaceutical products on the market. Among the mammalian cell lines available, human cells lines have emerged as the most promising alternative to substitute the main production platform based on murine CHO cells (Swiech et al., 2012). Human cell lines are capable of producing glycosylated proteins that are more similar to native human proteins, reducing the potential for immune responses against non-human epitopes.

Serum-free conditions are also extremely important considering that the expression of recombinant proteins with the use of serum-free components simplifies downstream processing and reduces the risks of xenogeneic contamination and development of immunogenic epitopes,

thereby improving the quality and safety of the protein produced. Moreover, the suspension culture reduces the costs of production, which is extremely important in biopharmaceutical industries. In this work, we evaluate two strategies to genetically modify the human cell lines Huh-7, HKB-11, SK-Hep-1, under serum-free suspension conditions, for recombinant protein production. Firstly, experiments were conducted to establish improved conditions for transient transfection and transduction using lentiviral vector encoding GFP expression. The best condition was then applied to genetically modify the cells for recombinant erythropoietin (rEPO) production.

2. MATERIALS AND METHODS

2.1. Cell Lines and Culture Conditions

The HKB-11, Huh-7 and SK-Hep-1 cell lines used were previously adapted for growth in serum-free suspension cultures by Biaggio et al, 2015. Suspension cell cultures were maintained in 125mL *erlenmeyer* flasks containing 20mL of culture medium under orbital stirring at 150 rpm in a humid atmosphere containing 5% CO_2 at 37°C. HKB-11 and SK-Hep-1 cells were routinely cultivated in FreeStyle 293 Expression Medium (Invitrogen) and Huh-7 in CD293 (Thermo Fisher).

2.2. Transient Transfection

The transient transfection protocol was performed using the lentiviral vector p1054-CIGWS encoding GFP expression, Polyethylenimine (PEIpro®, Polyplus Transfection) as a cationic donor and SFM4Transfx-293 (Hyclone) and FreeStyle (Invitrogen) serum-free media. Cells were transfected when density reached $1.0x10^6$ cells/mL in 12-well plate with a working volume of 1 mL with 1 µg DNA/10^6 cells and different DNA:PEI ratios (1:2, 1:3 and 1:4).

2.3. Lentiviral Transduction

Lentiviral particles were produced by transient transfection of HEK293F cell line using PEI (25 kDa linear PEI, Polysciences). The plasmids were transfected in the following ratio: 20 µg of the vector 1054 encoding GFP expression, 13 µg of vector pCMVΔ8.91 (containing HIV-1 gag, pol, rev and tat) and 7 µg of the vector pMD2 VSV-G (VSV-G envelope). Cells were cultured in DMEM 10% fetal bovine serum in static 75 cm^2 flasks. After 48 hours of culture, the supernatant was collected, centrifuged at 450 x g for 5 minutes at 4°C, filtered (0.45 µm filter) and stored at -80°C. Prior to transduction, the viral titer was determined by flow cytometry by measuring GFP expression. At the time of transduction, 1 mL of the cell suspensions was plated at $3x10^5$ cells/well in a 6-well plate. After addition of the viral supernatant containing 1 MOI (Multiplicity of infection), the cells were incubated at 37 ° C in a humid atmosphere containing 5% CO_2. After 72 hours the cells were collected for analysis of GPF expression as well as cell viability by flow cytometry. The same protocol was used for transduction of the human cells for rEPO production.

2.3. EPO Production Experiments

After genetic modification the cells were sorted by flow cytometry for selection of an homogenous population and then cultivated as previously described with an seeding density of $1x10^6$ cell/mL during a 3-days culture period. rEPO production was carried out using a biotin-avidin-amplified specific sandwich ELISA (Ab119522, Abcam) according to manufacturer's protocol.

5. RESULTS AND DISCUSSION

Figure 1A presents the results obtained for the transient transfection of the human cells lines under serum-free suspension conditions. The GFP expression levels obtained by transient transfection were not satisfactory, even testing different transfection conditions (different PEIPro:DNA ratios and different serum-free medium during transfection), except for HKB-11 cells transfected in FreeStyle. SK-HEP-1, HKB-11 and HUH-7 cells presented a maximum transfection efficiency of 7%, 49.5% and 4,8% (1:2 DNA:PEI ratio; FreeStyle serum-free medium), respectively.

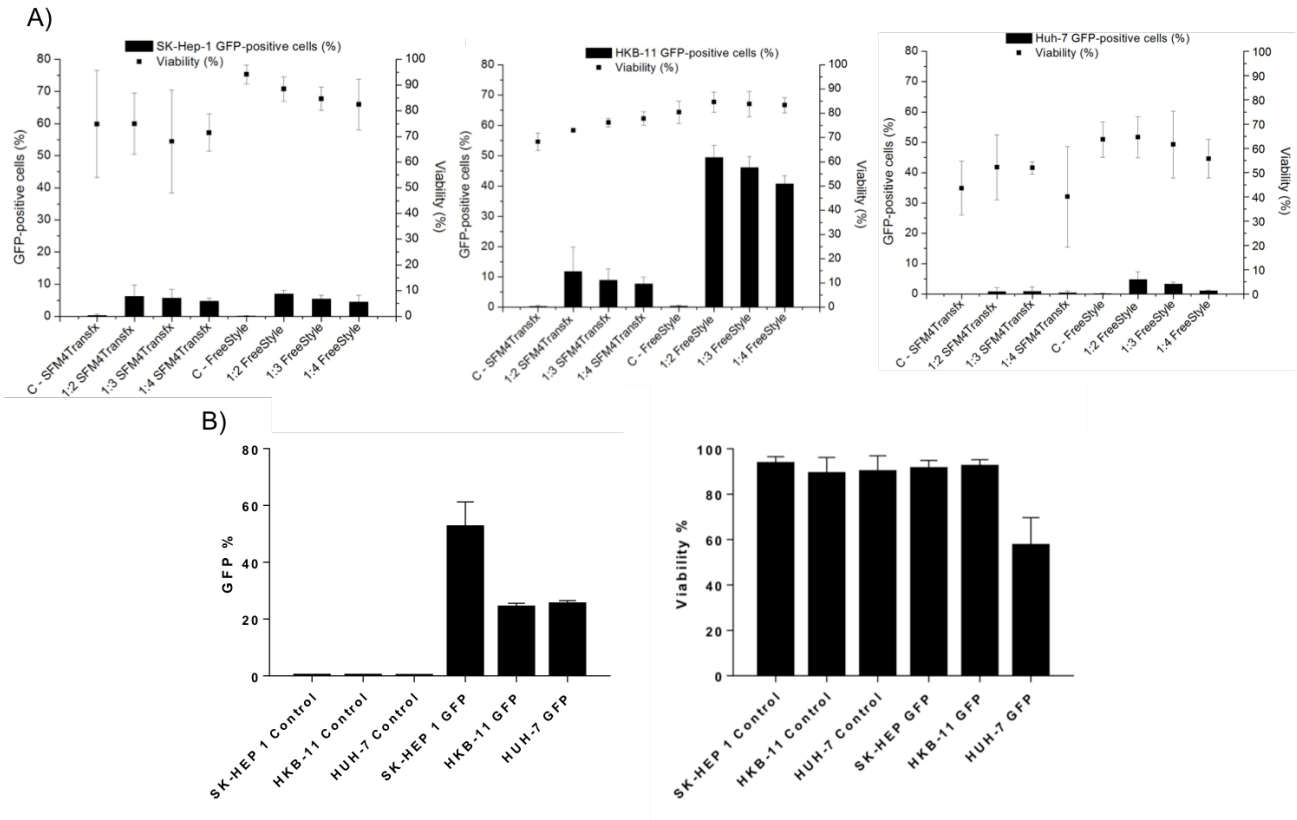

Figure 1. Genetic modification of the human cells SK-Hep-1, HKB-11 and Huh-7 under serum-free suspension conditions. A) Transient transfection using PEIpro and SFM4Transfx-293 and FreeStyle media (n=3). B) Lentiviral transduction with a MOI of 1 and FreeStyle medium (n=3). Control conditions represents non-modified cells.

By using lentiviral transduction (MOI of 1), it was possible to obtain satisfactory levels of GFP-positive cells without further optimization: 52.4, 26.4 and 24.8 for SK-Hep-1, HKB-11 and Huh-7 cells, respectively (Figure 1B). Based on this, genetic modification by transduction was chosen to generate the cells for rEPO production. After genetic modification (transduction efficiency of 50.8%, 63.1% and 33.7 for SK-Hep-1, HKB-11 and Huh-7 cells, respectively) and sorting (GFP expression of 99.0%, 95.0% and 90.0 for SK-Hep-1, HKB-11 and Huh-7 cells, respectively), the cells were cultivated for evaluation of rEPO production (Figure 2). After 72 hours of culture, SK-Hep-1, HKB-11 and Huh-7 produced 8.3; 16.5 and 94.6 µg/mL of rEPO. The levels of EPO expression achieved are compatible with those found in the literature (Sun et al., 2006; Yoon et al, 2005; Yoon et al, 2002). A full kinetic characterization for each cell line will be carried out to better evaluated the growth performance and EPO production rates.

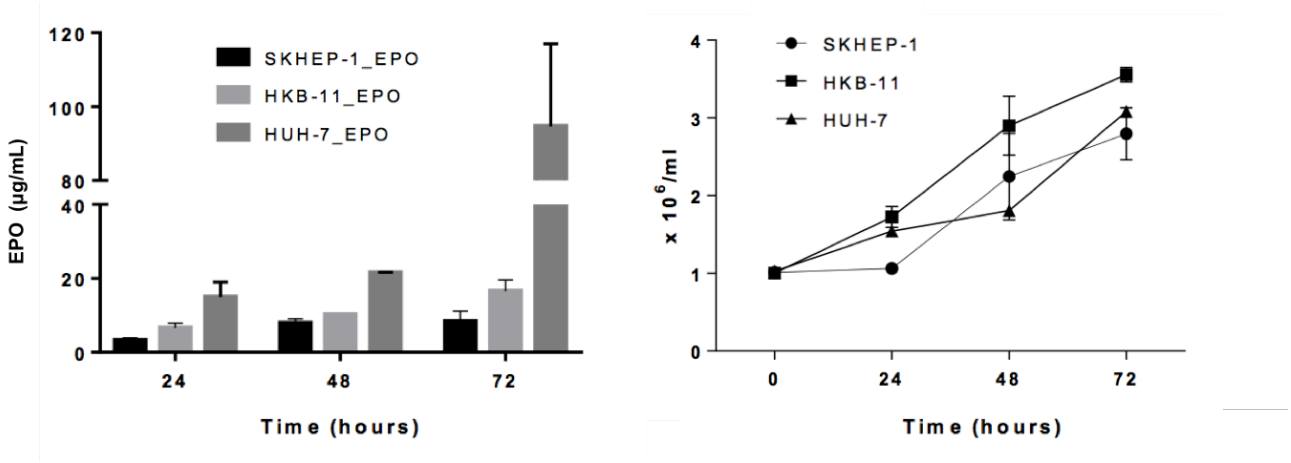

Figure 2. Recombinant EPO production and growth performance of the human SK-Hep-1, HKB-11 and Huh-7 cells cultured under SfS conditions (n=2).

The growing demand for mammalian-derived recombinant therapeutics proteins is driving the research and the development for optimized expression systems aiming at a high quality product with enhanced production yields.

5. REFERENCES

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