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Efficient Development of Stable Recombinant Chinese Hamster Ovary (rCHO) Cell Lines to Produce Antibodies by Using Dimethyl Sulfoxide (DMSO) in Electroporation

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Development of stable rCHO cell lines is still time consuming and labor intensive, although it is a critical step in the commercial development of recombinant antibodies. The current work demonstrates, for the first time, that electroporation of CHO cells with DMSO can enhance stable expression of recombinant antibodies in rCHO cells. Electroporation with DMSO resulted in an average 3.7-fold and 2.8-fold increases in expression levels of aflibercept and pembrolizumab, respectively, in pools of stable rCHO cells. It also resulted in an average of 2.2-fold and 2.6-fold increases in the expression of aflibercept and pembrolizumab, respectively, in single-cell derived rCHO clones. Simple batch cultures of rCHO cell clones with the highest expression produced 1.0 g/l for aflibercept and 1.4 g/l for pembrolizumab without a time-consuming gene amplification process. Electroporation with DMSO also shortened the development of rCHO cell lines to 2-3 months, allowing rapid establishment of stable rCHO cell lines with a desirable expression level antibodies.

Keywords: Dimethyl sulfoxide, stable cell line, Chinese hamster ovary cell, recombinant antibody, electroporation

Commercial production of recombinant proteins and antibodies on a large scale for therapeutic purposes requires the harmonized combination of various technologies. Among these, the technologies involved in the establishment of stable producer cell lines are critical for successful development of a manufacturing process for commercial production. Chinese hamster ovary (CHO) cells are currently the most widely used host cells for the production of recombinant proteins and antibodies for therapeutic purposes [1]. Thus, various technologies and equipment allowing rapid and inexpensive establishment of stable recombinant CHO (rCHO) cell lines have been developed in the past few decades [2–4]. However, the establishment of stable rCHO cell lines is still a time-consuming and labor-intensive process. It usually takes longer than six months, however, often over one year is needed when numerous steps are required in the gene amplification process with antibiotics to obtain desirable expression levels.

Efficient transfection methods of CHO host cells with the gene of interest is essential to establish stable rCHO cell lines. Transfection methods using cationic polymers and lipids have been developed for the transfection of adherent CHO host cells [5, 6] whereas the electroporation is widely used for transfection of suspension-adapted CHO host cells [7]. A number of compounds, so called transfection enhancers, can enhance transgene expression in various cell types when they are used as additives in transfection procedures [8]. Among these compounds, dimethyl sulfoxide (DMSO) can increase efficiency of transgene expression when it is used as an additive in electroporation of various host cells (HL60, TR146, Cos-7, and L132) [9]. DMSO can also enhance transient expression of transgenes in CHO cells [10]. However, advantages of using DMSO in transfection (electroporation) have not been reported for the establishment

of stable rCHO cell lines that can be used for commercial production of recombinant proteins and antibodies. The current work demonstrates that treatment of suspension-adapted CHO host cells with DMSO during electroporation can greatly facilitate the establishment of stable rCHO cell lines with high expression of antibodies.

First, cDNAs encoding heavy and light chains of two therapeutic antibodies, aflibercept [11] and pembrolizumab (GenBank Accession No. 5DK3_G and 5DK3_F), were synthesized and cloned separately under mouse EF1α promoter of pMmEG (TA) expression vector [12]. Suspension-adapted CHO DG44 (*dhfr*$^-$/*dhfr*$^-$) cells were used as host for antibody expression. These cells were cultured in HT-supplemented medium which was EX-CELL CD CHO (without hypoxanthine and thymidine) (Sigma-Aldrich) supplemented with 4 mM L-glutamine (Sigma-Aldrich) and 1X HT supplement (Thermo Fisher Scientific) and harvested. Then, 3 μg of aflibercept expression plasmid or a mixture of 1.5 μg heavy chain and 1.5 μg light chain expression plasmids for pembrolizumab was mixed with 22 ng of pDCH1P [13] containing the *dhfr*$^+$ gene. This DNA solution was mixed with 6×10^5 CHO host cells. Electroporation was performed with or without 1.25% DMSO by using Neon Transfection System 10 μl Kit (Thermo Fisher Scientific) and Microporator MP-100 (Thermo Fisher Scientific). The optimal concentration of DMSO in electroporation was determined to be 1.25% from the experiments with different concentrations (data not shown). Transfectants obtained were pooled and grown in HT-supplemented medium for 5 days and transferred to a selection medium that was EX-CELL CD CHO (without hypoxanthine and thymidine) supplemented with 4 mM L-glutamine for two weeks in order to select stable rCHO cells.

These pools of stable rCHO cells were expanded in selection medium and seeded at 1×10^5 cells/well in 1 ml of selection medium in a 12-well plate and grown for 4 days. Quantitation of antibody in culture supernatant was then performed by ELISA established by using goat anti-human IgG (Fc specific) antibody (Sigma-Aldrich) and goat anti-human IgG Fc Cross-Adsorbed Secondary Antibody (Thermo Fisher Scientific). Fig. 1 shows antibody expression in pools of stable rCHO cells obtained from electroporation in the presence or absence of DMSO. DMSO used in electroporation resulted in an average of 3.7-fold increase in aflibercept expression (Fig. 1A) and an average of 2.8-fold increase in pembrolizumab expression (Fig. 1B). Therefore, DMSO used in electroporation significantly increased the stable expression of antibodies.

The effects of DMSO on the stable expression of antibodies were also evaluated in single-cell derived rCHO cell clones. These single-cell derived rCHO cell clones were isolated from pools of rCHO cells by limiting dilutions (0.5 cells/well) in 0.2 ml of selection medium in multiple 96-well plates. These cells were grown for 4 weeks and the single-cell clonality was confirmed by continuous monitoring with Cell Metric CLD (Solentim Ltd.). After quantitating the expression of all rCHO cell clones by using ELISA, 10 clones for each antibody were selected from the highest antibody expression. These clones were expanded in selection medium and seeded at 1×10^5 cells/ml in 1 ml of selection medium in a 12-well plate. These cells were grown for 4 days and quantitation of antibody in culture supernatant was performed by ELISA. Fig. 2 shows antibody expression in individual rCHO clones. DMSO used in electroporation resulted in an average of 2.2-fold increase in aflibercept expression (Fig. 2A) and an average of 2.6-fold increase in the expression of pembrolizumab

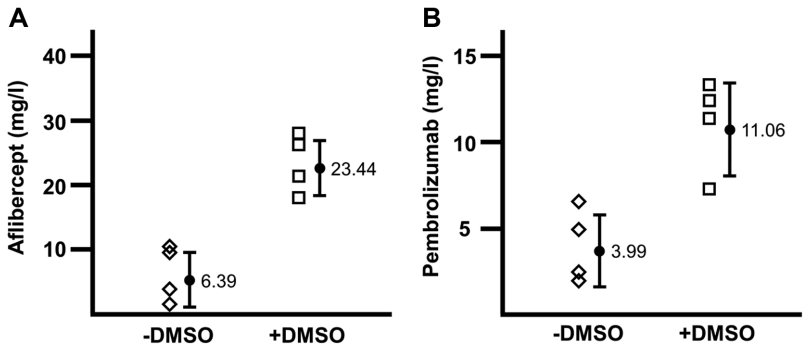

Fig. 1. Expression of antibodies in pools of stable rCHO cells.

Four independent electroporations were performed for aflibercept (**A**) or pembrolizumab (**B**) in the absence (open rhombuses) or presence (open rectangles) of DMSO. Average expression (numbers) and standard deviations (error bars) were calculated from quantitation of the accumulated antibody proteins in culture supernatants.

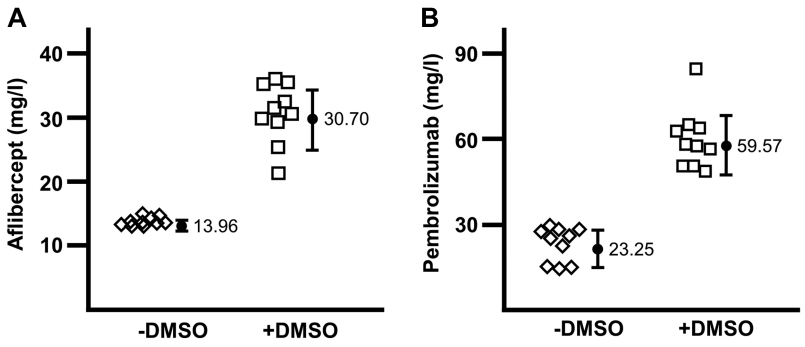

Fig. 2. Expression of antibodies in single-cell derived rCHO cell clones.

The top ten rCHO cells from the highest expression for aflibercept (**A**) and pembrolizumab (**B**) were selected by limiting dilution after electroporation in the absence (open rhombuses) or presence (open rectangles) of DMSO. Average expression (numbers) and standard deviations (error bars) were calculated from quantitation of accumulated antibody proteins in culture supernatants.

(Fig. 2B). Therefore, increase in antibody expression in single-cell derived rCHO cell clones by using DMSO in electroporation was confirmed.

The single-cell derived rCHO cell clones with the highest expression were selected for aflibercept and pembrolizumab (Fig. 2). Their expression levels were evaluated by establishing simple batch cultures. The simple batch cultures were performed in 250 ml Erlenmeyer flasks with 90 ml HyCell CHO (GE Healthcare Life Sciences) medium supplemented with 4 mM L-glutamine and 10 ml CD EfficientFeed C AGT Nutrient Supplement (Thermo Fisher Scientific). The seeding density was at 1.0×10^6 cells/ml. Cultures were performed at 34°C, and 1 ml of 7.5% sodium bicarbonate solution was added to these cultures when the pH of the culture went down below 6.8. Antibody proteins accumulated in the culture medium were analyzed by ELISA. Both aflibercept and pembrolizumab accumulated in the culture supernatant on Day-12 of the simple batch cultures were 1.0 g/l (Fig. 3A) and 1.4 g/l (Fig. 3B), respectively.

The current work for the first time demonstrates the effect

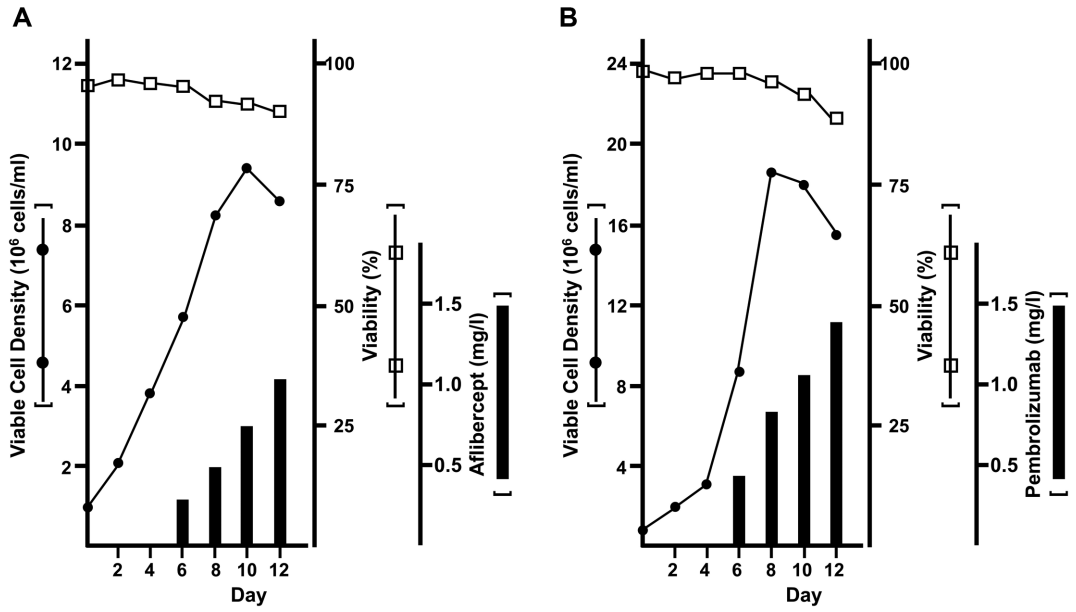

Fig. 3. Expression profiles of simple batch cultures.

rCHO cell clones with the highest expression for aflibercept (**A**) or pembrolizumab (**B**) were selected and cultured in 250 ml Erlenmeyer flasks (100 ml working volume). Viable cell density (-●-), cell viabilities (-□-), and levels of accumulated antibodies in the culture medium (filled rectangle) are indicated.

of using DMSO in electroporation on the stable expression of antibodies in rCHO cells. Stable expression of two therapeutically important antibodies, aflibercept and pembrolizumab, was enhanced several times both in pools of stable rCHO cells and in single-cell derived rCHO cell clones by using DMSO in electroporation, resulting in efficient and rapid development of stable rCHO cell lines for commercial development. These stable rCHO clones could be obtained within 2-3 months after electroporation with DMSO. The resulted stable rCHO clones showed productivity over 1 g/l in simple batch culture conditions. Furthermore, these stable rCHO cells with a desirable production rate can be established without a time-consuming gene amplification process using antibiotics known to cause instability of expression during long-term cultures of rCHO cells [14]. Although further optimizations of medium, feeding strategy, and bioreactor operation are needed to establish a manufacturing process with higher productivity for commercial purposes, the results in this study are applicable to rapid establishment of stable rCHO cell lines for developing antibodies as therapeutics. The role of DMSO in facilitating DNA uptake during transfection has been well described in transient expression of antibodies [10]. However, DMSO might have additional roles in enhancing the stable expression of antibodies in rCHO cell lines.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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