Bioprocess and Biosystems Engineering (2019) 42:1263–1271
https://doi.org/10.1007/s00449-019-02124-7

RESEARCH PAPER

Bioprocess development of a stable *FUT8*$^{-/-}$-CHO cell line to produce defucosylated anti-HER2 antibody

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Received: 3 January 2019 / Revised: 3 April 2019 / Accepted: 4 April 2019 / Published online: 13 April 2019

Abstract

In recent years, an increasing number of defucosylated therapeutic antibodies have been applied in clinical practices due to their better efficacy compared to fucosylated counterparts. The establishment of stable and clonal manufacturing cell lines is the basis of therapeutic antibodies production. Bioprocess development of a new cell line is necessary for its future applications in the biopharmaceutical industry. We engineered a stable cell line expressing defucosylated anti-HER2 antibody based on an established α-1,6-fucosyltransferase (*FUT8*) gene knockout CHO-S cell line. The optimization of medium and feed was evaluated in a small-scale culture system. Then the optimal medium and feed were scaled up in a bioreactor system. After fed-batch culture over 13 days, we evaluated the cell growth, antibody yield, glycan compositions and bioactivities. The production of anti-HER2 antibody from the *FUT8* gene knockout CHO-S cells in the bioreactor increased by 37% compared to the shake flask system. The *N*-glycan profile of the produced antibody was consistent between the bioreactor and shake flask system. The antibody-dependent cellular cytotoxicity activity of the defucosylated antibody increased 14-fold compared to the wild-type antibody, which was the same as our previous results. The results of our bioprocess development demonstrated that the engineered cell line could be developed to a biopharmaceutical industrial cell line.

Keywords Bioprocess development · CHO cells · Defucosylated antibodies · Fed-batch · Stable cell line

Introduction

Therapeutic monoclonal antibodies (mAb) are currently used in the treatment of many diseases, such as cancer [1], inflammatory disorders, infectious diseases and genetic disorders [2]. Over the past two decades, monoclonal antibodies and their related products have dominated the biopharmaceutical market. In order to ensure adequate supplies of these recombinant proteins for growing market demands, researchers are working to understand and engineer cell expression systems better. Establishing a clonal cell line with high productivity and optimal growth is basic and important work. There are two approaches applied, including identification of a high-producing cell line and optimization of the cell culture process [3].

Once a new cell line is developed, cell culture process development aims to fully maximize the cell line's potential productivity and to maintain product quality by optimizing cell culture medium and feed formulation, culture strategies and process parameters such as pH, temperature, dissolved oxygen and osmolality [4].

Several different bioreactor processes have traditionally been developed to maximize mAb production. Methods range from simple batch and fed-batch culture to more complex continuous culture or perfusion culture [5]. Among these methods, perfusion culture is suitable for sensitive protein products because of the short product residue time [3, 6]. For relatively stable products, fed-batch culture has become the most widely used method in cell line bioprocess development due to its ease of operation, flexibility and fast development. In fed-batch process, feed medium is supplied to the cell culture with a fixed volume at regular intervals during cultivation.

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Therapeutic mAbs mediate their antitumor effects through two major mechanisms, including complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) [7]. It has been reported that a deficiency of fucose from the carbohydrate chain attached to Asn297 on the human IgG1 Fc region could improve the affinity with FcγRIIIa receptor on NK cells and enhance ADCC bioactivity [8–10].

Chinese hamster ovary (CHO) cells have been utilized extensively for therapeutic protein production because of correct folding and authentic posttranslational modifications [11]. The deletion of α-1,6-fucosyltransferase (*FUT8*) enzyme could make CHO cells produce defucosylated mAb [12, 13].

In our previous work, an *FUT8* knockout ($FUT8^{-/-}$) CHO-S cell line was generated using the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9) technologies [14, 15]. In this report, we engineered an $FUT8^{-/-}$-CHO-S cell line stably expressing defucosylated mAb and attempted to develop a fed-batch culture strategy in bioreactors by bioprocess development. We verified the biological activity of the products. Our study provides a fed-batch culture strategy to produce defucosylated antibodies with higher productivity and equivalent quality, which will contribute to scale-up the process from a laboratory to the industry manufacturing in the future.

Materials and methods

Cell transfection and clone screening

FUT8 knockout CHO-S ($FUT8^{-/-}$-CHO-S) cell line was engineered in our previous study [14, 15]. Humanized anti-HER2 antibody (Herceptin, Drug Bank: DB00072) was selected as the model protein. Antibody heavy chain (HC) and light chain (LC) encoding regions were cloned into vector pIRES separately, followed by insertion of an anti-puromycin gene. The two vectors pIRES-HC-puro and pIRES-LC-puro were co-transformed into $FUT8^{-/-}$-CHO-S cells using electroporation at 1800 V for 20 ms with 1 pulse. Transfected cells were cultured in CD-CHO medium (Gibco, Carlsbad, CA, USA) supplemented with 8 mM GlutaMax at 37 °C, 8% CO_2 and shaken at 125 rpm. After 48 h, 16 μg/mL of puromycin (Amresco, Solon, OH, USA) was used for screening cells. After two rounds of limiting dilution, high-expression single clones were selected with Western blot assessment.

LCA labeling assay

Cells were seeded at a density of 3×10^5 cells/mL and stained with 15 μg/mL fluorescein-labeled *Lens culinaris* agglutinin (FITC–LCA; Reactolab SA, Servion, Switzerland) for 30 min according to the manufacturer's manual, and then were subjected to flow cytometric analysis (Beckman Coulter, IN, USA).

Cell culture media and feeds screening

Five serum-free commercial media were used for media screening in this study (Table 1). Three highest expression cell lines were cultured in the five media supplemented with 8 mM GlutaMax, respectively. Cells were cultured in an incubator (ThermoFisher Scientific, Shanghai, China) controlled at 37 °C and 8% CO_2, at 125 rpm. Cell density and viability were measured daily using a Trypan Blue (Sigma-Aldrich, St. Louis, MO, USA) dye exclusion method. When cell viability dropped below 60%, the produced mAb was purified for productivity determination and glycosylation analysis. Then the cell line and medium with highest productivity were chosen for feed screening. Three feeds were used in this study (Table 1). Feed was added on days 3, 5, 7, 8 and 9 with 5% (v/v) feeding volume. The cultured conditions were as described above.

Bioreactor culture conditions

Bench-scale fed-batch cell culture processes were conducted in a 3-L BioFlo/CelliGen 310 benchtop bioreactor (Eppendorf, Shanghai, China). Selected cell line was seeded at a density of 2×10^5 cells/mL controlled at 37 °C, 100 rpm with 50% dissolved oxygen (DO) and pH 7.0. Feed was administered on days 3, 5, 7, 8 and 9 with 5% (v/v) feeding volume. Medium was collected daily for measurements of viable cell density, viability and productivity until cell viability dropped below 60%. Metabolite concentration and osmolality were

Table 1 Selected commercial media and feeds for screening

Types	Products	Manufacturer	Catalog no.
Medium	SFM4CHO	HyClone	SH30548.02
	CDM4CHO	HyClone	SH30558.02
	IS CHO-CD	Irvine Sientific	94110
	CD-CHO	Gibco	10743029
	Forti CHO	Gibco	A1148301
Feed	CHO Feed 4	Irvine Scientific	94134
	Cell Boost 6	Hyclone	SH30866.01
	Efficient Feed A	Gibco	A1023401

also determined using a Nova Bioprofile 400 analyzer (Nova Biomedical, Waltham, MA, USA).

Analysis of protein concentration

The purification was performed using an AKTA purifier system (GE Healthcare, Uppsala, Sweden). Harvested culture medium containing mAb was loaded on a HiTrap rProtein A FF column (GE Healthcare) [16] with Buffer A (20 mM phosphate, 150 mM NaCl, pH 7.2) as a loading buffer and buffer B (100 mM sodium citrate, pH 3.0) for elution. The collected fractions were neutralized with 1 M Tris–HCl (pH 9.0) and then quantitated by BCA assay.

Glycosylation analysis based on HILIC–HPLC

Glycosylation analysis of the anti-HER2 antibody was conducted on an Agilent 1260 HPLC system (Aligent Technologies, Shanghai, China). The 2-aminobenzamide (2-AB) labeled *N*-glycan standards (G_0, G_0F, G_1, G_1F, G_2, G_2F; ProZyme, Hayward, CA, USA) were used as control. N-linked oligosaccharides were cleaved by PNGase F (New England Biolabs, MA, USA) and labeled with 2-AB labeling reagent (Prozyme) at 65 °C for 3 h, then the excess labeling reagent was removed by hydrophilic interaction liquid chromatography-solid phase extraction cartridges (HILIC SPE; Waters, Shanghai, China). The purified 2-AB-labeled *N*-glycans were dried and redissolved in 60% acetonitrile in water (v/v) for HPLC analysis.

Mobile phase A was 10 mM NH_4AC in 80:20 (v/v) acetonitrile (ACN)/water and B was 10 mM NH_4AC in 50:50 (v/v) ACN/water. *N*-Glycan samples were separated on a SeQuant ZIC-HILIC column (5 µm, 200A, 250 × 4.6 mm; Merck, Darmstadt, Germany). Gradient separation was conducted at 35 °C with a flow rate of 1 mL/min and the analytes were detected with a fluorescence detector set at an excitation wavelength of 330 nm and an emission wavelength of 420 nm. The elution gradients were as follows: 0 min, 20% B; 10 min, 35% B; 35 min, 60% B; 36 min, 100% B; 42 min, 100% B; and 43 min, 20% B, which was continued for 17 min for column equilibration [17].

ADCC assay

The HER2-overexpressing breast tumor cell line BT-474 and peripheral blood mononuclear cells (PBMCs) were used to study the toxicity of defucosylated (Her-*FUT8*$^{-/-}$) and wild-type (Her-WT) anti-HER2 antibodies. PBMCs were isolated from human blood by Ficoll-Hypaque density gradient separation. BT-474 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Invitrogen, Shanghai, China). The target cells (BT-474, 2×10^4 cells/well in 96-well plates) were pre-incubated with various concentrations of antibodies for 30 min in 37 °C in RPMI 1640 medium supplemented with 5% FBS, and then added the effector cells (PBMCs) in a 10:1 *E*/*T* ratio and incubated for an additional 20 h. The supernatants were harvested for detection of lactate dehydrogenase activity using a CytoTox 96 non-radioactive cytotoxicity kit (Promega, Shanghai, China) [18–20]. Each reaction was performed in triplicate and percent cytotoxicity was calculated using following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental} - \text{effector spontaneous} - \text{target spontaneous}}{\text{Target maximum} - \text{target spontaneous}} \times 100.$$

Western blotting

Samples of culture supernatant were loaded into a 10% gel and transferred to polyvinylidene difluoride (PVDF) membranes at 200 mA for 90 min. The membranes were blocked with 10% skimmed milk and incubated with peroxidase-conjugated donkey anti-human IgG antibody (H + L; Jackson Immunoresearch, West Grove, PA, USA). Target antibodies were detected using Immobilon Western Chemiluminescent HRP Substrates (Millipore, Bedford, MA, USA).

Results

Establishment of stable anti-HER2 antibody expressing cell clones

FUT8$^{-/-}$-CHO-S cells were co-transfected with vectors pIRES-HC-puro and pIRES-LC-puro using electroporation. Under the pressure selection of puromycin, surviving cells were seeded at 0.5 cell/well in 96-well plates for limited dilution. Two rounds of limited dilution were applied to ensure the monoclonality of selected clonal cell lines. After 3 weeks of incubation, 12 high-expression cell clones were selected by dot blot from 106 clones and transferred into 24-well plates in the first round. At the same time, protein expression of colonies was analyzed by Western blot. The second round of limited dilution was conducted with the same method. Finally, three high-expression single cell-derived sub-clones (cell line 4, cell line 9 and cell line 10) were selected by Western blot (Fig. 1a, b) from transfected pools and named Her-*FUT8*$^{-/-}$-CHO-S cells.

The absence of α-1,6-fucosylation in N-linked oligosaccharides in selected *FUT8* knockout clones could be further confirmed by the affinity of LCA to cell surface fucose [13]. With wild-type CHO-S cells as control, we tested the three selected sub-clones using FITC–LCA staining methods.

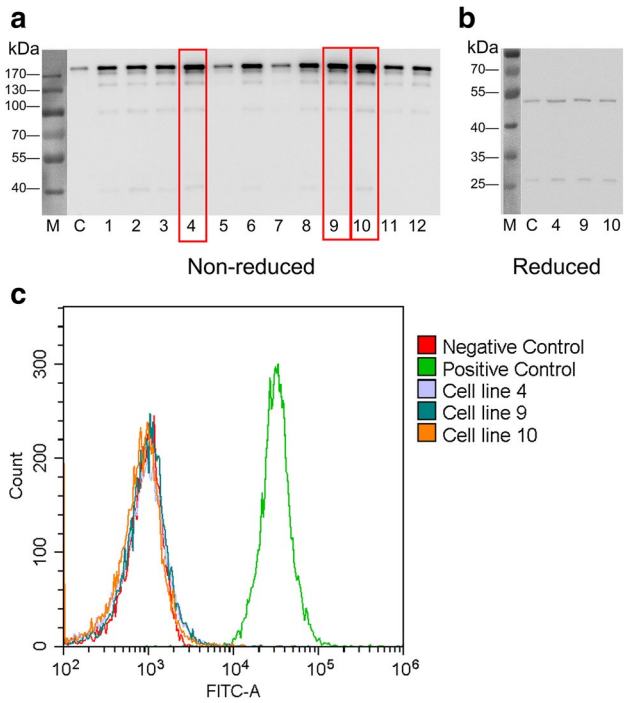


Fig. 1 Selection of stable anti-HER2 antibody expressing cell clones. Anti-HER2 mAb expression in supernatants were detected by Western blot. **a** Non-reduced proteins of 12 cell clones. **b** Reduced proteins from 4 selected high-producing clones. *M* Marker. *C* Positive control (25 ng/mL of anti-HER2 antibody). 1–12: Selected cell clones. **c** FITC-LCA staining of fucose on wide-type CHO-S and selected FUT8 knockout CHO-S cell clones. Positive control: Wild-type CHO-S stained with FITC-LCA. Negative control: Wild-type CHO-S without stained. 1–3: Selected *FUT8*$^{-/-}$-CHO-S sub-clones stained with FITC-LCA

Negative binding of LCA indicated that the *FUT8* gene was completely absent in the three sub-clones (Fig. 1c).

Medium and feed optimization for high expression cell line

In order to screen the optimal combination of cell line and culture medium, three selected cell clones were evaluated in a batch culture process with five commercial media as described in the Materials and Methods. There were eight groups (cell line 4 in SFM4CHO medium, cell line 4 in CDM4CHO medium, cell line 4 in IS CHO-CD medium, cell line 9 in SFM4CHO medium, cell line 9 in CDM4CHO medium, cell line 9 in IS CHO-CD medium, cell line 10 in SFM4CHO medium and cell line 10 in IS CHO-CD medium) eventually survived, and their cell density and productivity are shown in Table 2 and Fig. 2a. Based on batch culture, we concluded that SFM4CHO medium was better than other media according to the productivity observed. At the same time, cell line 9 showed higher expression than the others, with a peak cell density of 4.68×10^6 cells/mL and the highest mAb production of 28.13 mg/L/day when cultured in SFM4CHO medium.

For biopharmaceuticals, proper glycosylation is a crucial quality concern due to its significant effects on protein therapeutic efficacy. All *N*-glycan samples and standards were analyzed by an HPLC-fluorescence method and well separated using gradient elution as shown in Fig. 2b. Compared with standards, the negative signals of fucosylated *N*-glycans (G_0F, G_1F and G_2F) indicated that the Her-*FUT8*$^{-/-}$-CHO-S cell lines produced defucosylated antibody (Fig. 2c). The relative abundances (%) of the labeled *N*-glycans were calculated by computing the area of each peak, producing a percentage of the total peak area of all glycans in the sample. The results revealed that medium had a limited impact on glycosylation, the products from eight groups showed a similar glycan distribution (Fig. 2d).

To further improve the mAb expression in bioproduction, we evaluated three kinds of feeds with cell line 9 and SFM4CHO medium in fed-batch culture. The measurements were similar to those of the media screening, and feeding strategy was designed based on the manufacturer's protocol. Cell line 9 cultured in SFM4CHO without feed supplement was the control group. The feeds listed in Table 1 were added separately to the culture on day 3.

In this study, while the addition of the three feeds facilitated the cell growth, extended the culture duration and increased mAb productivity, CHO Feed 4 (Table 1)

Table 2 Selection of cell lines and medium for culture process

Cell line	Media	Groups	Culture time (days)	Peak viable cell density (10^6 cells/mL)	Productivity (mg/L/day)
Cell line 4	SFM4CHO	1	7	4.97	26.95
	CDM4CHO	2	6	3.56	10.69
	IS CHO-CD	3	8	3.20	9.46
Cell line 9	SFM4CHO	4	6	4.68	28.13
	CDM4CHO	5	6	4.49	12.74
	IS CHO-CD	6	8	4.49	11.56
Cell line 10	SFM4CHO	7	6	4.05	23.76
	IS CHO-CD	8	8	3.68	9.16

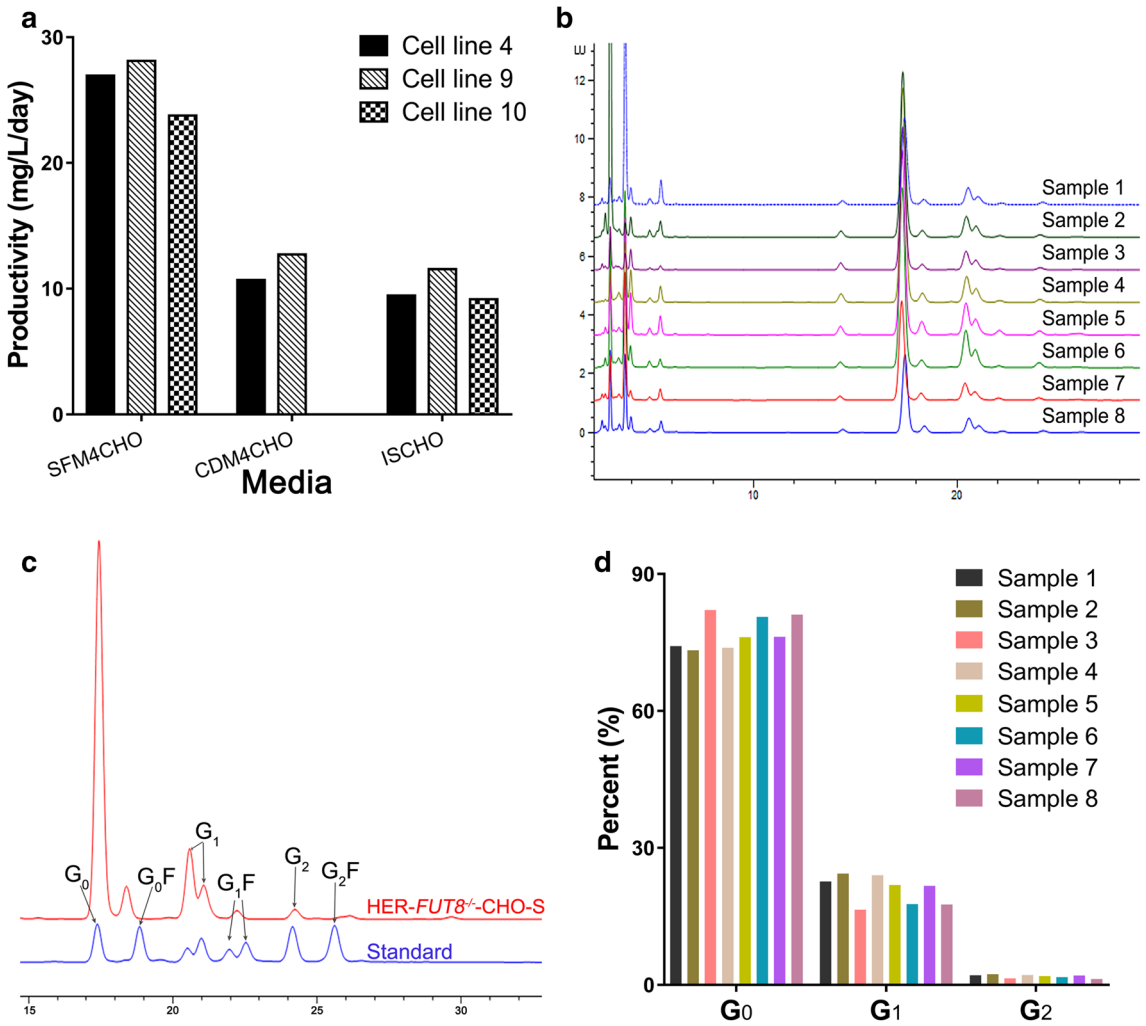


Fig. 2 Cell line and medium screen. **a** Comparison of productivity between different cell lines and media. **b** Hydrophilic HPLC chromatography of *N*-glycans. Samples 1–8 represent eight groups, respectively. **c** *N*-glycans analysis. **d** Relative abundance of major glycans. Sample 1: cell line 4 in SFM4CHO medium. Sample 2: cell line 4 in CDM4CHO medium. Sample 3: cell line 4 in IS CHO-CD medium. Sample 4: cell line 9 in SFM4CHO medium. Sample 5: cell line 9 in CDM4CHO medium. Sample 6: cell line 9 in IS CHO-CD medium. Sample 7: cell line 10 in SFM4CHO medium. Sample 8: cell line 10 in IS CHO-CD medium

was found having a more dramatic effect than the others (Fig. 3). The peak cell density was found to be 19.4×10^6 cells/mL on day 10 (Fig. 3a), whereas no more than 13.5×10^6 cells/mL was reached when cells were supplemented with Cell Boost 6 (Table 1). The product yield reached up to 581.5 mg/L, which was 3.5 times that the control group (Fig. 3c).

According to the data of *N*-glycan analysis data (Fig. 3d), the relative abundance of G_2 varied from 0.692% (efficient Feed A) to 2.126% (CHO Feed 4). The yield of antibody produced with addition of efficient Feed A ranked second after CHO Feed 4, while its glycan profile indicated a low level of glycosylation. Thus, CHO Feed 4 was selected as the optimal feed due to its high productivity and desirable glycan profile.

Scale-up culture in benchtop bioreactor

The combination of SFM4CHO medium and CHO Feed 4 was scaled up from 200 mL to 3 L in a benchtop bioreactor. Cells were maintained in fed-batch mode with pH controlled at 7.0. Sampling was performed daily and subjected to cell count and metabolic analysis. The profiles of cell growth and major metabolites are summarized in Fig. 4.

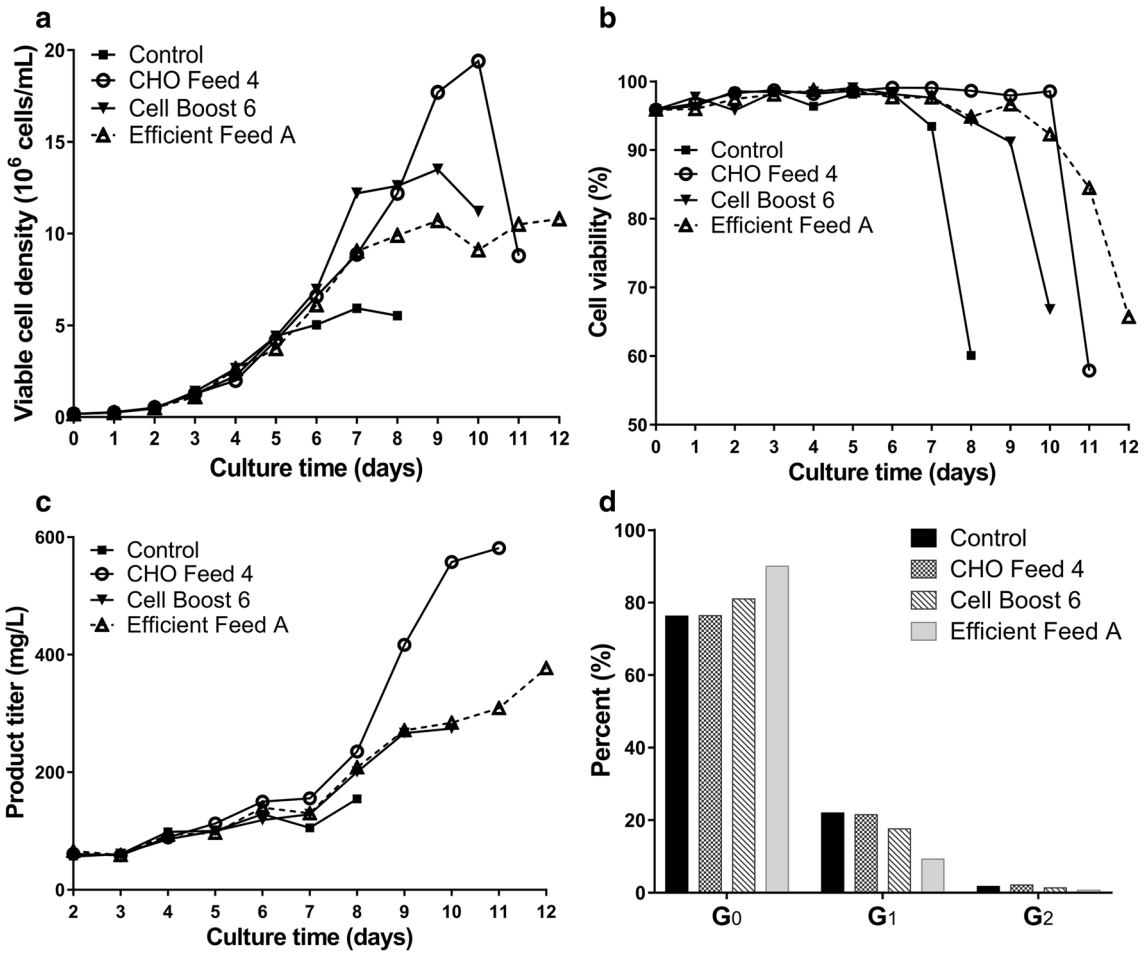


Fig. 3 Feed screen. Three fed-batch cultures for cell line 9 with different feeds. **a** Viable cell density, **b** cell viability, **c** product titer and **d** relative abundance of major glycans

During the whole culture process, osmolality increased continuously, but remained below 380 mOsm/kg (data not shown). A rapid consumption of glucose was observed during the exponential growth phase of cells, from 7.8 to 4.83 g/L on day 3, then the consumption rate slowed down and finally the glucose level tended to stabilize round 3 g/L. As a byproduct of glucose, 2.48 g/L of lactate was produced during the exponential phase and then was gradually consumed down to 1.03 g/L (Fig. 4c). Cells were undergoing a metabolic switch from lactate production to lactate consumption when the glucose was at a low level. The glutamate concentration increased with fluctuations, and reached 5.71 mM at the end of culture. Ammonia accumulation continued over time, reached 13.81 mM at the end (Fig. 4d), presumably due to the metabolism of glutamine or ammoniagenic amino acids.

The growth profile was illustrated in Fig. 4a. The viable cell density in the bioreactor was lower than predicted, with a peak viable cell density of 6.19×10^6 cells/mL on day 11 and then dropped to 3.12×10^6 cells/mL at the end of the culture. The viability remained above 90% until day 10, but then sharply dropped. The cumulative product yield increased along with culture time and reached the highest product yield of 797.17 mg/L on day 13 (Fig. 4b). There was a 37% increase compared to the culture in the flask (581.3 mg/L).

To evaluate whether *N*-glycan distribution changed during the scale-up culture, the *N*-glycan profile of the antibody from the bioreactor was compared with that from the flask. As shown in Table 3, the glycan composition profile was quite comparable between the samples from the bioreactor and the flask. Thus, the growth of cells in bioreactors allowed a higher titer of production and retained a desirable product quality.

The ADCC activities of purified anti-HER2 antibody

To evaluate the ADCC activity of the anti-HER2 antibody produced by *FUT8*$^{-/-}$-CHO-S cell lines (Her-*FUT8*$^{-/-}$), anti-HER2 antibody from wild-type CHO-S cells (Her-WT)

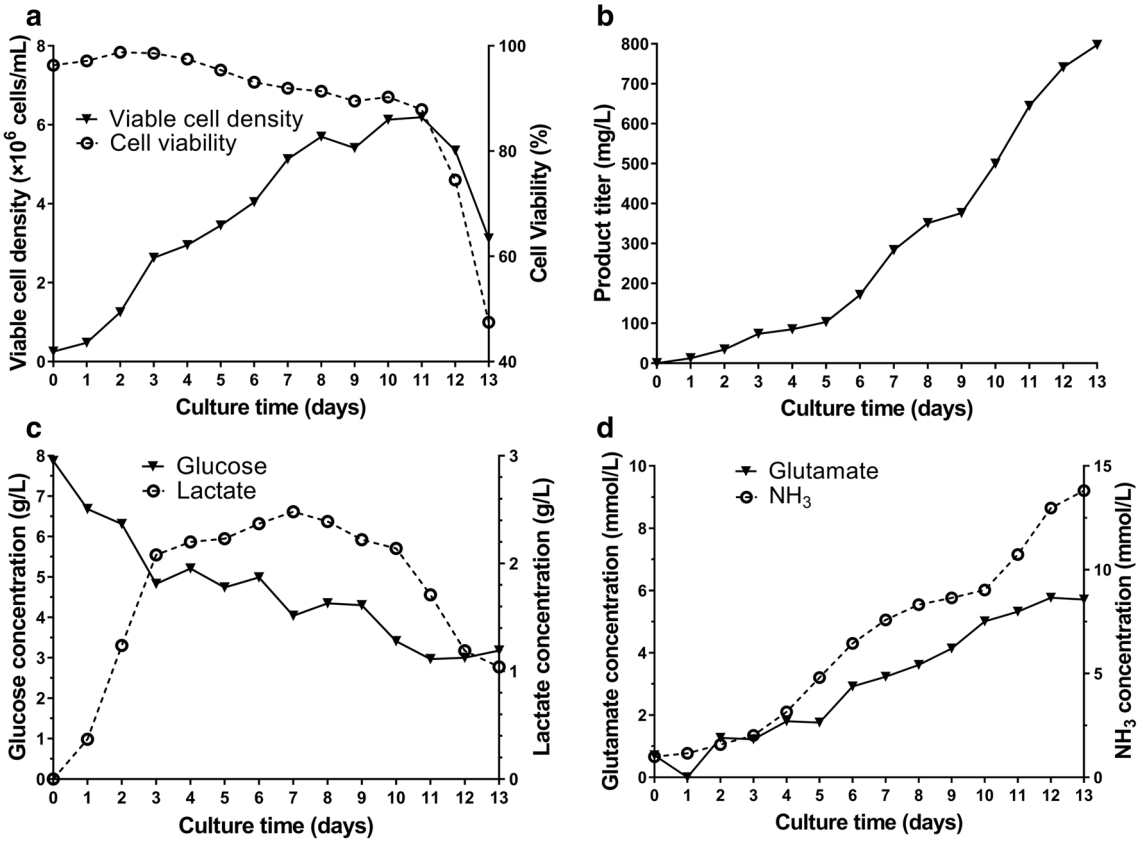


Fig. 4 Fed-batch culture in bioreactor. Cell line 9 cultured in SFM4CHO medium with CHO Feed 4. **a** Viable cell density and cell viability. **b** Product titer. **c** Profiles of glucose and lactate concentrations. **d** Profiles of glutamate and ammonia concentrations

Table 3 *N*-Glycan profile of antibodies produced in flask and bioreactor

Sample	G_0 (%)	G_1 (%)	G_2 (%)
Shake flask	76.383	21.492	2.126
Bioreactor	82.850	15.960	1.190

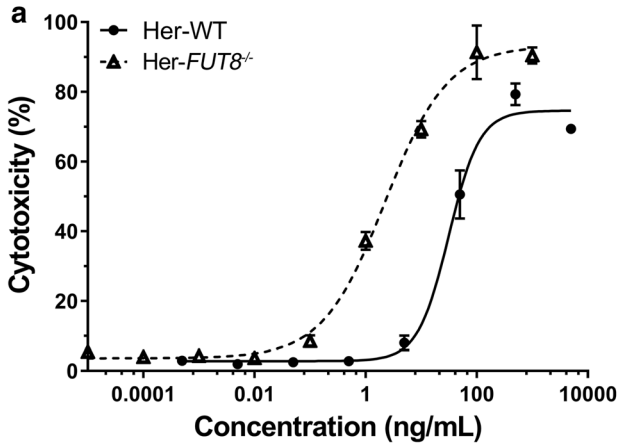
was used as control. The results showed that defucosylated anti-HER2 antibody had a higher cytotoxicity than wild-type fucosylated anti-HER2 antibody (Fig. 5), more specifically, the ADCC activity increased about 14-fold.

Discussion

The demand for therapeutic antibodies based on mammalian cells continues to increase. To obtain biopharmaceuticals at an economic cost, increased attention has been paid to the large-scale mammalian cell culture. It is believed that establishment of high-productivity cell line and optimization of culture processes, especially the culture medium and feed, are key elements to improve mAb production. To engineer a host cell line that produces defucosylated mAbs with superior ADCC activities, we have previously disrupted the α-1, 6 fucosyltransferase (*FUT8*) gene in CHO-S cells by CRISPR/Cas9 technology [14, 15]. In current study, we further developed this cell line for potential industrial applications.

In bioprocess development, three primary factors impact culture performance, product expression and product quality: the production cell line, the cell culture medium and feed, and the process parameters. Today, a variety of commercial cell media and feeds are available, but none is universal for all production systems because each specific production cell line has different nutrient requirements. It is necessary to optimize the culture medium and feed for a new cell line.

First, we selected high-producing stable cell lines and confirmed that the antibodies they produced were completely defucosylated. In medium and feed screening, cell line 9 with medium SFM4CHO and CHO Feed 4 were determined to be the optimal combination. The peak viable cell density was 19.4×10^6 cells/mL and the final titer reached up to 581.5 mg/L in the flask system. The results showed



b

Target cell	Antibody	Maximal lysis (%)	Minimal lysis (%)	R^2	EC_{50} (ng/mL)	Ratio
BT-474	Her-*FUT8*$^{-/-}$	93.14	3.62	0.992	2.24 ± 0.62[a]	14: 1
	Her-WT	74.58	2.83	0.987	31.44 ± 8.25	

Fig. 5 ADCC activities of defucosylated and wild-type anti-HER2 antibodies. **a** ADCC activities on BT-474 cells at different mAb concentrations. **b** LDH release assay. Cytotoxicity measurements were done in triplicate and data are shown as mean ± standard deviation (SD). **a** $*p < 0.05$

that designing optimal medium and feed for a specific cell line could significantly improve mAb production with the desired protein quality.

In the mammalian cell culture process, glucose and glutamine generally serve as the main carbon and energy sources. The metabolism of glucose in most mammalian cell lines (including CHO cells) is carried out by glycolysis. Glucose is converted to lactate via aerobic glycolysis, which is energy inefficient, generating only 2 ATP compared with 36 ATP when glucose is completely oxidized to CO_2 in TCA cycle [21, 22]. In our study, we noticed that glucose was consumed over time, while the metabolism of lactate shifted from excretion to consumption in the later stage of culture. Our results were identical to the previous CHO metabolic studies [23, 24]. In the presence of high glucose levels, cells consumed glucose and produce lactate. When glucose was consumed down to a low concentration, the cells started to consume lactate, which is a more energy efficient type of metabolism [25]. In our study, glutamate and ammonia were excreted throughout the culture period due to their direct connection to glutaminolysis, where glutamine is lysed to glutamate and ammonia [26, 27]. Lactate and ammonia are well-known inhibitors of cellular growth. During the culture, lactate concentration was kept under control at less than 2.5 g/L (2.48 g/L), which seemed to have a minimal impact on the culture process. The total generation of 13.8 mM ammonia impacted the culture greatly because a significantly inhibition of cell growth was observed in 10 mM ammonia [28].

Bioreactors are widely used for commercial manufacturing of biopharmaceuticals in mammalian cell systems [29], and are designed to support the optimal cell growth. Bioreactor systems can control the culture in a more precise way than the shake flask system. Tight controls of process parameters, such as pressure, temperature, pH, dissolved oxygen, nutrient supply, and liquid evaporation all contribute to a much better cell growth and production yield. The high degree of reproducibility, automation and process control introduced by bioreactors has been key for their application in large-scale production in the modern biopharmaceutical industry [30]. Stirred tank bioreactors are a conventional type of reactors used in the industry and have been used for over 40 years [31, 32]. They are also suitable for smaller operations, such as experimental development, because of their versatility, flexibility, and low operating costs.

In this study, fed-batch cell culture method was used to evaluate the new engineered CHO cell line, and the culture time and productivity of cells were dramatically improved in the scale-up culture in a bioreactor system. Culture time was extended to 13 days, and cumulative yield was increased by 37%. The results showed that we developed the fed-batch process which was effective for the stable *FUT8*$^{-/-}$-CHO cell line to produce defucosylated anti-HER2 antibody in the scale-up culture. Poor viability was originally observed. We considered that this problem might have been due to the inhibition of hydrodynamic shear forces and excessive ammonia concentration. These issues would be improved greatly after adopting the culture strategy described in the future experiments. To ensure the product quality, we conducted characterization analysis on the products. The *N*-glycans analysis showed that the glycan profile of the antibody produced in the bioreactor was consistent with that of the flask. We also tested the ADCC activities of the products. The defucosylated antibodies showed superior ADCC activities compared with wild-type fucosylated antibody for HER2-overexpressing cancer cells.

In summary, we have developed the bioprocess of the engineered *FUT8* knockout CHO-S cell line with the higher productivity than that from previous shake flask study and consistent quality. However, the influences of excessive accumulation of ammonia and hydrodynamic shear forces on cell growth cannot be ignored. Effective strategies should be adopted in future studies to improve culture performance and maximize cell productivity for future industrial purposes.

Acknowledgements This project was supported by National Science Foundation of China [Grant number: 81502969].

Compliance with ethical standards

Conflict of interest All the authors reviewed and agreed to submit this manuscript. The authors declare that they have no conflict of interest.

Research involving human participants and/or animals The study does not contain experiments using animals and human studies.

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