Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–9, 2017

www.elsevier.com/locate/jbiosc

Development of hyper osmotic resistant CHO host cells for enhanced antibody production

Yasuharu Kamachi[1,2,*] and Takeshi Omasa[3,4]

Takeda Pharmaceutical Company Limited, 26-1 Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan,[1] Graduate School of Advanced Technology and Science, Tokushima University, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan,[2] Faculty of Bioscience and Bioindustry, Tokushima University, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan,[3] and Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan[4]

Received 3 September 2017; accepted 6 November 2017
Available online xxx

Cell culture platform processes are generally employed to shorten the duration of new product development. A fed-batch process with continuous feeding is a conventional platform process for monoclonal antibody production using Chinese hamster ovary (CHO) cells. To establish a simplified platform process, the feeding method can be changed from continuous feed to bolus feed. However, this change induces a rapid increase of osmolality by the bolus addition of nutrients. The increased osmolality suppresses cell culture growth, and the final product concentration is decreased. In this study, osmotic resistant CHO host cells were developed to attain a high product concentration. To establish hyper osmotic resistant CHO host cells, CHO-S host cells were passaged long-term in a hyper osmotic basal medium. There were marked differences in cell growth of the original and established host cells under iso- (328 mOsm/kg) or hyper-osmolality (over 450 mOsm/kg) conditions. Cell growth of the original CHO host cells was markedly decreased by the induction of osmotic stress, whereas cell growth of the hyper osmotic resistant CHO host cells was not affected. The maximum viable cell concentration of hyper osmotic resistant CHO host cells was 132% of CHO-S host cells after the induction of osmotic stress. Moreover, the hyper osmotic resistant characteristic of established CHO host cells was maintained even after seven passages in iso-osmolality basal medium. The use of hyper osmotic resistance CHO host cells to create a monoclonal antibody production cell line might be a new approach to increase final antibody concentrations with a fed-batch process.

[**Key words:** Chinese hamster ovary cells; Osmotic resistant; Cell growth; Antibody production; Hyper-osmolality]

Chinese hamster ovary (CHO) cells are widely used as a host to produce various therapeutic proteins including recombinant monoclonal antibodies. A fed-batch cell culture process with concentrated nutrient feeding throughout the culture period is commonly used for the industrial scale production of therapeutic proteins. There are two types of feeding strategy for fed-batch cultures: (i) continuous feeding; and (ii) bolus feeding. The fed-batch process with continuous feeding is suitable for maintaining exact nutrient concentrations during cell culture by controlling the feed rate of feed medium based on the consumption of nutrients. Therefore, cells in a fed-batch process with continuous feeding will have less stress from environmental conditions including osmolality during the culture period compared with bolus feeding. Currently, the fed-batch process with bolus feeding is the most widely used method for industrial large-scale mammalian cell culture because of its simple operation, reduced facility requirements and inexpensive manufacturing. Therefore, it is important to develop a simplified platform process with simple operation that meets GMP manufacturing standards, which will reduce the risk of human error and contamination. High productivity of the platform process is also important to meet increasing market demand and reduce the cost of goods. To improve productivity, various approaches have been developed for cell and cell culture processes, i.e., construction of highly-productive cell lines (1–11), effective strategies for cell line selection (12,13), improved feeding methods (14–17), optimization of cell culture media (18–23), usage of perfusion seed culture (24), environmental parameters such as pH (25), temperature (26), and dissolved oxygen (22). In general, the final concentration of a recombinant protein in a fed-batch culture is affected by the viable cell density, viable cell culture longevity and specific production rate of the recombinant protein. Osmolality strongly influences these three important parameters. It is well known that hyper osmotic pressure suppresses cell growth. Zhu et al. (27) showed that an increase of osmolality resulted in a linear reduction of the specific growth rate and led to a 60% decrease at 450 mOsm/kg compared with control medium at 316 mOsm/kg (27). However, hyper osmotic stress increases specific productivity in CHO cell lines (28) and hybridoma cell lines (29). Wu et al. (29) reported a decrease in the growth rate and an increase in the specific production rate of hyper osmotic (450 mOsm/kg) cultures compared with iso-osmotic (290 mOsm/kg) cultures. Omasa et al. (30) reported that the inhibition of hybridoma cell growth was mainly caused by osmotic pressure while lactate production from glucose was inhibited by lactate itself

* Corresponding author at: Takeda Pharmaceutical Company Limited, 26-1 Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan. Tel.: +81 466 32 4347; fax: +81 466 29 4420.
E-mail address: yasuharu.kamachi@takeda.com (Y. Kamachi).

1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2017.11.002

because of an increase in osmotic pressure. Furthermore, increased osmotic pressure increased the productivity of hybridoma cultures (29,30). However, an increase in the specific production rate does not result in a substantial increase of the final product concentration because of depressed cell growth and decreased cell viability (28,29). A substantial increase of specific productivity with an increase of osmolality was observed in a batch culture in response to significant metabolic changes. Specific glucose and glutamine consumption rates were increased in a dose-dependent manner in batch cultures. However, the final product concentration did not substantially increase because of a decrease in viable cell density (28). Shen et al. (28) also reported that hyper osmolality slightly repressed the growth rate of fed-batch cells and no significant change in productivity and final product concentration was observed. Several studies have reported intracellular changes in response to osmotic stress. In one study, over 600 genes related to cell cycle distribution, growth, transcription and translation regulation in the GS-N0 cell line were affected by hyper osmotic conditions during cell culture (29). However, few studies have investigated the development of host cells that do not suppress cell growth in hyper osmotic cultures.

In a previous study, a fed-batch process with continuous feeding for monoclonal antibody (IgG) production by CHO cells was developed for pilot plant scale (data not shown). This platform process maintained high cell density and produce high antibody concentrations. However, a more simplified process is required for industrial production to reduce the risk of microbial contamination and operation failure by human error. Therefore, we developed a new bolus feeding method instead of conventional continuous feeding. The bolus feeding method lead to a greater increase in medium osmolality compared with the conventional continuous feeding method because of the large amount of feed volume required to be added in a single administration. Higher osmolality in the culture with the bolus feed method might have a negative effect on cell growth. In this study, hyper osmotic resistant CHO host cells were developed to overcome growth suppression effects by hyper osmotic pressure. To establish these hyper osmotic resistant CHO host cells, an adaptation method with hyper osmotic medium was applied. Batch cultures under osmotic stress conditions were used to confirm the osmotic resistance of the hyper osmotic resistant CHO host cells with CHO-S host cells as controls. Detailed kinetic and metabolic analyses were performed, and stability of the hyper osmolality resistance of the osmotic resistant CHO host cells was evaluated.

MATERIALS AND METHODS

Cell line and cell culture conditions of the bioreactor A serum-free adapted recombinant GS-CHO cell line producing human monoclonal antibody was grown in a fed-batch culture. Fed-batch cultures were grown in a 5-L bioreactor (Sartorius, NY, USA) with a 3-L working volume. A 5-L bioreactor was equipped with a pitched blade (diameter = 7.0 cm). The bioreactor temperature was maintained at 37 °C, and the agitation speed was 400 rpm. A gas mixture containing air and O_2 was provided. The dissolved oxygen concentration (DO) was controlled using an on-line feedback control at 30 mmHg by varying the oxygen pressure in the gas mixture. Air was supplied through the headspace of the bioreactor for CO_2 removal. pH control was performed by sparging with CO_2 gas into the gas mixture or the addition of 7.5% $NaHCO_3$ solution into the medium.

Protein-free chemical defined in-house media were used for the fed-batch culture. For the fed-batch operations, continuous feeding or bolus feeding methods were used. In continuous feeding, feed medium containing glucose and nutrients was continuously fed based on the glucose concentration to maintain the glucose concentration at a target level of 2 g/L. In the bolus feeding method, the same feed medium was used based on the glucose concentration once every two or three days to maintain a glucose concentration of >2 g/L.

Cell line and cell culture conditions of shake flasks CHO-S host cells (Life Technologies, CA, USA) were used to develop osmotic resistant CHO host cells. In the fed-batch study, we used GS-CHO cells, but we changed the CHO host cell to CHO-S for the adaptation experiments because of the limitations of the host cell license. CD-CHO medium (Life Technologies) containing 8 mM glutamine and NaCl were used to develop osmotic resistant CHO host cells using repeated batch cultivation and developed CHO cell lines were evaluated using batch cultivation. Culture media with different osmolalities were prepared by varying the amount of NaCl added. A sterile 2 M NaCl solution was added into medium to increase the osmolality to the target level. For batch culture evaluation, CHO-S and osmotic resistant CHO cells in the exponential growth phase were inoculated at 2.0×10^5 cells/mL into a 125 mL shake flask with a 40 mL working volume and the time-course of cell growth was analyzed. The cells were cultured at 37 °C and 125 rpm in a humidified 5% CO_2 incubator.

Viable cell density, viability, productivity and osmolality Viable cell density and viability were measured using Vi-Cell Automated Cell Counters (Beckman Coulter, CA, USA). Antibody concentration was measured using Protein A HPLC. Medium osmolality was measured by Vapor Pressure Osmometer (Wescor, UT, USA) for the fed-batch cultures and by an osmometer (Arkray Osmostat OM-6040, Arkray, MN, USA) for the batch cultures. Both osmometers used the freezing point method for measurement.

RESULTS AND DISCUSSION

To establish a simplified platform cell culture method, the feeding method was changed from continuous feed to bolus feed. Fig. 1 shows the comparison between continuous and bolus fed-batch cultures. Recombinant GS-CHO cell line A producing human monoclonal antibody was used in both cultures. The continuous feed and bolus feed began at 44 h after inoculation to maintain a glucose concentration >2 g/L. Glucose was selected as a surrogate to represent the overall nutrient consumption. In the continuous feed culture, the feed rate was determined by the glucose consumption rate and the pre-determined next sampling time was based on maintaining a glucose concentration between 2 and 3 g/L. In the bolus feed culture, a fixed feed volume was added when the glucose concentration was predicted to be <2 g/L at the day after sampling. The glucose concentration was maintained between 2 and 10 g/L (Fig. 1A). GS-CHO cell line A with the continuous feeding method reached stationary phase at 187 h after inoculation and a maximum cell concentration of 19.6×10^6 cells/mL, whereas with the bolus feeding method cell growth was suppressed after 115 h and the viable cell concentration was unchanged until 211 h after inoculation. The maximum cell concentration was 11.1×10^6 cells/mL with the bolus feeding method (Fig. 1B). The maximum cell concentration of the GS-CHO cell line A with bolus feeding was 55% of the control culture with continuous feeding. The culture osmolality gradually increased in the continuous feed culture (Fig. 1C) to >440 mOsm/kg at 115 h and 524 mOsm/kg at 335 h after inoculation. A significant difference in viable cell density between the continuous and bolus fed-batch cultures was observed at 115 h after inoculation. However, there was no difference in the viability of the GS-CHO cell line A with continuous feeding or bolus feeding throughout the culture period. The viability was 82.1% for continuous feeding and 76.9% for bolus feeding at 355 h (Fig. 1D). The integral viable cell concentration (IVC) at 355 h after inoculation was 2.04×10^8 cells/mL for the continuous feeding method and 1.11×10^8 cells/mL for the bolus feeding method (Fig. 1E). The IVC with bolus feeding was 54% of the control culture with continuous feeding. The cell-specific productivity, q_p, was calculated as the antibody concentration divided by the IVC over the culture duration (Fig. 1F). The q_p at 355 h was 1.49 pg/cell/hour for the continuous feeding method and 2.08 pg/cell/hour for the bolus feeding method. These results showed that increased osmolality in the bolus feed method led to a 40% increase in q_p at 355 h compared with the control culture with continuous feeding. However, the increase of q_p did not result in a substantial increase in the final mAb concentration. The final monoclonal antibody (mAb) concentrations in the bolus-fed and continuous-fed cultures were 5.6 g/L and 7.3 g/L, respectively (Fig. 1G). The final mAb concentration was 77% of the control culture product concentration

Please cite this article in press as: Kamachi, Y., and Omasa, T., Development of hyper osmotic resistant CHO host cells for enhanced antibody production, J. Biosci. Bioeng., (2017), https://doi.org/10.1016/j.jbiosc.2017.11.002