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Characteristics of human cell line, F2N78, for the production of recombinant antibody in fed-batch and perfusion cultures

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A human hybrid cell line, F2N78, was developed by somatic fusion of HEK293 and Namalwa cells for the production recombinant biopharmaceutical proteins. In this study, we performed perfusion culture to verify its potential in culture process used for human cell expression platform. Cell viability could be maintained over 90% and high viable cell density was obtained at higher than 1.0×10^7 cells/mL by bleeding process in perfusion culture. The cells were adapted well in both culture modes, but there were apparent differences in protein quality. Compared to fed-batch culture, degalactosylated forms such as G0F and G0 as well as Man5 showed no significant increases in perfusion culture. In terms of charge variants, acidic peaks increased, whereas main peaks constantly decreased according to the length of culture period in both methods.

[**Key words:** Human cells; F2N78 cells; Fed-batch culture; Perfusion culture; Glycosylation]

Recombinant antibodies have been used as major therapeutics for treating cancer and rheumatoid arthritis. More than 30 antibodies were approved for various medical applications up to date. Antibodies are complex glycoproteins with large molecular size. To manufacture therapeutic antibodies, an expression platform based on a recombinant cell line is usually employed. For the production of recombinant glycoproteins, mammalian cells are the most preferred host due to their capability to synthesize target glycoproteins that are similar to natural counterparts in human body (1). Especially, an expression system from rodent is the most preferred for producing target antibodies. Chinese hamster ovary (CHO) and baby hamster kidney (BHK) cells, which are derived from hamsters, as well as NS0 and SP2/0 (myeloma) cells, which are derived from mice, are usually employed for manufacturing recombinant therapeutic proteins.

Because of the issues on immunogenicity derived from rodent cells, utilization of human cells as new host cells has been attempted (2). Human embryonic kidney cells (HEK293) were used for the production of Xigris (activated protein C) (3). HKB11, a hybrid cell line of HEK293 and a human B cell line, was developed for the production of cytokines (4). Potential of CAP cell line derived from human amniocytes was reported for influenza virus production in suspension culture (5). Another human cell line, PER.C6 derived from primary culture of human fetal retinoblast, was generated to produce recombinant antibodies (6) and human adenoviral vectors (7). Furthermore, the F2N78 cell line was recently established as a new human expression system via fusion of HEK293 and Namalwa cells. F2N78 cells constantly express EBNA-1 protein, which interacts with OriP-utilized vector for improvement of transient gene expression. This novel host cell line can produce recombinant proteins in both transient and stable manners (8).

Not only for the development of host cells, but also evaluation of culture mode is an important part of establishing production system (1). In the field of biopharmaceuticals, fed-bath and perfusion culture strategies have been used as the dominant processes in mammalian cell cultures (9). Development of a stable fed-batch process to enhance the productivity of target protein is a primary step in using host cells for the production of biopharmaceuticals. Fed-batch process was dominantly utilized for industrial production of recombinant biologics (10). Perfusion cultures were also employed to increase productivities and prolong culture period (11). However, the use of perfusion culture has been limited for a long time due to the complexity of the process as well as technical uncertainty. In recent years, perfusion culture strategies for overcoming these negative issues have been developed such as spin-filter technology (12). Spin-filter system in perfusion culture is the most adopted approach in commercial processes due to its simplicity and convenience in continuous culture (13).

Aggregation propensity of HEK293 cells was reported to be originated from several factors such as calcium ion concentration (14). Because the parental HEK293 cell line employed in fusion of F2N78 cells was not adapted for suspension culture, aggregation could be a problem in long-term culture of F2N78 cells. In addition, perfusion culture is a complicated strategy that requires at least 25 days with many factors that could stimulate aggregation, so that

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exclusion of aggregation is necessary. Another objective of this study is the prevention of ammonia accumulation, as glutamine was totally depleted while F2N78 cells constantly produced ammonia in suspension culture. Ammonia is toxic to most mammalian cells and shows negative effects such as reduction of cell density and alteration of protein quality (15). To inhibit excessive accumulation of ammonia, a modified culture process was developed.

In this work, we performed fed-batch and perfusion cultures to evaluated performance of a novel host cell line expression system, F2N78 cells, in both culture modes. Protein quality in each culture process was monitored for comparison.

MATERIALS AND METHODS

Cell line and culture medium Recombinant F2N78 cell line producing CT A IgG was made into a preliminary clone by stable transfection. Transfection was performed using FreeStyle293 MAX reagent (Life Technologies, USA). After stable transfection, puromycin selection was conducted. Limiting dilution cloning (LDC) for selection of single cell clones was not carried out. Expression vector contained IgG heavy and light chain genes as well as puromycin *N*-acetyl-transferase (PAC) gene as a selection marker. The promoter for expression of heavy and light chains was CMV promoter. The promoter for the selection marker was SV40 promoter. F2N78 cells were cultivated in EX-CELL293 (SAFC Biosciences, USA) supplemented with 2 μg/mL of puromycin (SFAC) and 4 mM glutamine (Thermo Fisher Scientific, Waltham, MA, USA). F2N78 cells were cultivated in a 125-mL Erlenmeyer flask (Corning, USA) at 110 rpm and 37°C, 5% CO_2.

Perfusion culture Perfusion culture was performed using a 3-L stirred tank bioreactor (Applikon, Netherlands) and spin-filter with a 15–17 μm pore size for cell retention. The F2N78 CT A TF5-2-17 clone was inoculated at a density of 0.5×10^6 cells/mL into a bioreactor with a working volume of 1.8 L. Basal medium was EX-CELL293 with 4 mM glutamine and 6 g/L of glucose. On post-inoculation day 3, perfusion was initiated. A Watson Marlow pump (Falmouth, UK) was used to supply media, harvest cell culture fluid, and for bleeding. Rotation speed of Watson Marlow pump at 0.1 rpm is equal to 262.08 mL/day. In terms of media change rate, In/Out/Bleed means the 'media inlet rate/media outlet rate/bleeding rate'. During the entire culture period, the media change rate increased from 0.2 to 0.58 v/v/d. In growth phase (day 0–7), EX-CELL293 with 4 mM glutamine and 6 g/L of glucose was supplied to the bioreactor. The production phase that we hypothesized was the culture period after day 7 when viable cell density was reached to 1×10^7 cells/mL. In production phase, a mixture of feeding medium composed of 85% EX-CELL293 with 4 mM glutamine and 10 g/L of glucose and 15% FMC006 (Celltrion, Korea) was used until the end of culture.

To maintain a constant viable cell density (VCD) and inhibit clogging of the spin-filter, cell bleeding was performed using a pipe located in the culture vessel. When VCD reached at $1.0–1.5 \times 10^7$ cells/mL, bleeding rate was adjusted at 524.16 mL/day (0.2 rpm). When VCD was over 1.5×10^7 cells/mL, bleeding rate was increased to 786.24 mL/day (0.3 rpm) or 1048.32 mL/day (0.4 rpm).

Anti-foam was added up to 0.03% of the working volume at day 8. Volume of the anti-foam was increased up to 0.05% until foam was vanished. Concentration of dissolved oxygen was controlled at 40% using a drilled hole sparger. The pH level was regulated at 7.0 with a dead zone of 0.05 by CO_2 sparging and 1 N NaOH, and the temperature was controlled at 37°C. Perfusion culture was performed for 30 days.

Fed-batch culture Fed-batch culture was performed in a 3-L stirred tank reactor. The F2N78 CT A TF5-2-17 clone was inoculated at a density of 0.5×10^6 cells/mL into a bioreactor with a working volume of 1.8 L. Concentration of dissolved oxygen was controlled at 40% using a drilled hole sparger. The pH level was regulated at 7.0 with a dead zone of 0.05 by CO_2 sparging and 1 N NaOH, and the temperature was controlled at 37.0°C. Fed-batch culture was performed for 17 days. Basal medium was EX-CELL293 with 4 mM glutamine and 10 g/L of glucose. FMC006 feed medium was fed every 5% of initial working volume (v/v) on day 3, 5, 7, 9 and 11. Glucose concentration was monitored and controlled at 5 g/L. Feeding was carried out on days 3, 5, 7, 9, and 11.

Analysis Sampling was performed daily during culture. Glucose, glutamine, lactate, and ammonia concentrations were analyzed using a Nova Bioprofile analyzer (Nova BioMedical, USA). Viable cell concentration and viability were measured by using a Vi-cell automatic cell counter (Beckman Coulter, USA). CT A protein was quantified using rProtein A HPLC. Osmotic pressure was analyzed using a Varpro 5600 osmometer (Wescor, USA).

Distribution of charge isoform Distribution of charge isoforms of CT A antibody was analyzed by cation ion-exchange chromatography HPLC (IEC-HPLC). A Propac WCX-10 analytical column (4 mm × 250 mm; Dionex, USA) and Propac WCX-10G guard column (4 mm × 50 mm) were coupled to the HPLC system (Waters, USA)

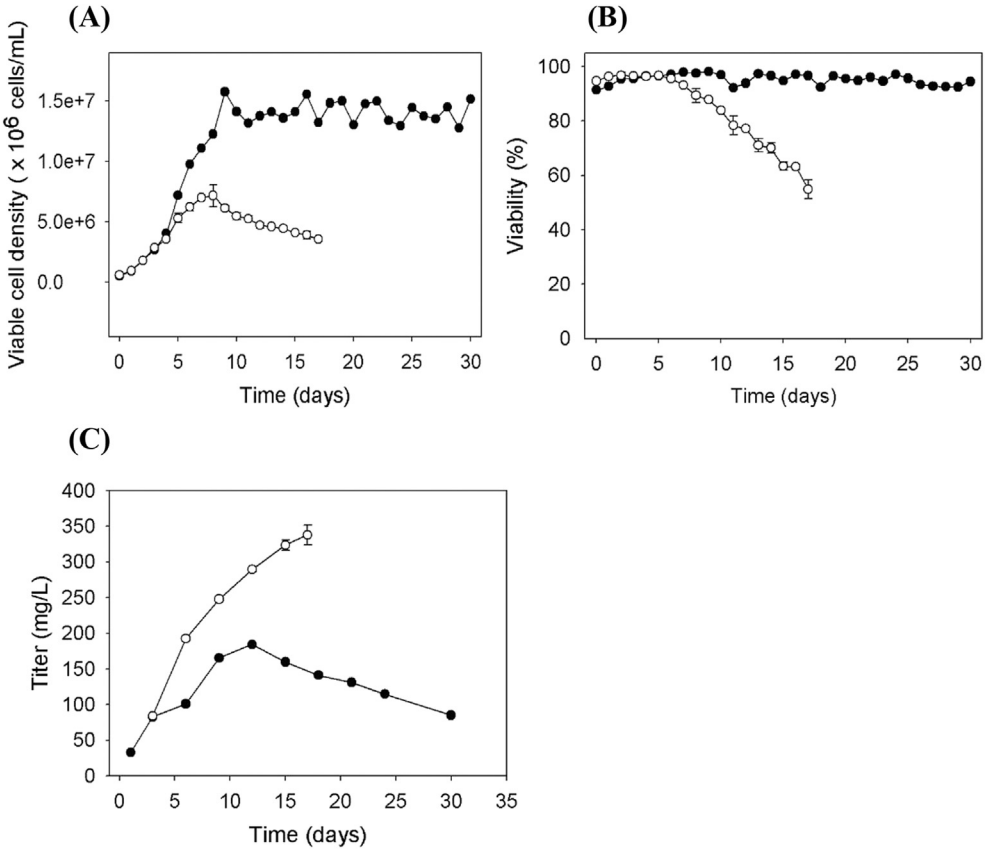

FIG. 1. Time course profiles of viable cell density (A), viability (B), and IgG titer (C) during perfusion (closed circles) and fed-batch culture (open circles).