A STUDY AND PRODUCTION OF MONOCLONAL ANTIBODIES THRU HYBRIDOMA TECHNOLOGY

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Abstract

The hybridoma 192 had previously been grown only in serum containing media, mostly in static flasks. Baseline data were obtained in tissue culture flasks (T-flask) with the cells grown in Dulbecco Modified Eagle’s Medium (DMEM) supplemented with 5% fetal bovine serum (FBS). Cells were first adapted to grow in DMEM with a reduced serum content of 2%. These adapted cells were grown in T-flasks, spinner flasks and a 2 L stirred tank bioreactor, to assess process scalability. Compared to T-flasks and spinner flasks, a high specific MAb production rate could be achieved in the highly controlled conditions of the 2 L bioreactor.

INTRODUCTION

The main objective of this study was to develop and scale-up a submerged culture process for the production of a diagnostic monoclonal antibody (MAb) against Congenital Adrenal Hyperplasia (CAH) using the hybridoma 192 cell line. The intention was to establish a process that could provide a substantial quantity of the Mab inexpensively. Studies included measurement of the baseline data in static culture and the bioreactor; optimization of a low serum medium with inexpensive supplements; identification of possible inducer agents; operational parameters optimization in the bench scale bioreactor using a design of experiment approach; and scale up to 20 L bioreactor. A summary of the outcomes of the study and its main conclusions are provided in the following sections.

Congenital adrenal hyperplasia (CAH) is an inherited genetic disorder that is due to the autosomal recessive effects in cortisol biosynthesis. CAH results in glucocorticoid and mineralocorticoid deficiencies and an increased level of adrenocorticotropic hormone (ACTH). ACTH then induces adrenal hyperplasia and overproduction of the steroids that result in various manifestations of the disorder. CAH is associated with defects in enzymes 4 and 5 in steroidal biosynthesis pathway shown in Figure 1.1 (Friedman, 2004).

Classic CAH can usually be diagnosed at birth or during childhood. It can be further divided into the salt-wasting (SW) form and the simple virilising (SV) form. Life-threatening salt-loss crisis, hyponatraemia, hyperkalaemia, dehydration, shock and ambiguous genitalia in females, are the characteristics of SW form. The SV form can be recognized through pseudo-precocious puberty in males and different degree of clitoris hypertrophy and posterior labial fusion in females (Votava et al., 2005). Only one third of classic CAH cases are of SV form; the rest are SW form (Friedman, 2004).

Non-classic or late-onset CAH manifests during or after puberty. It is easier to diagnose in female than male due to the hirsutism and cycle irregularities that are obvious. Even though this type of CAH is not life threatening, it causes accelerated
bone maturation that result in a reduced final height (Votava et al., 2005).

**Rationale for using monoclonal antibodies (MAb)**

The current ELISA method for diagnosis of CAH is based on polyclonal antibody (González et al., 2008). Use of MAb based assays for detection has many advantages, especially in its specificity that lowers the risk of a false positive diagnosis. In addition, a MAb is a well-defined protein that possesses uniform characteristics as compared to polyclonal antibody. A mouse-mouse hybridoma (hybridoma 192) capable of producing a monoclonal antibody for detecting CAH had been developed by the Physiology Department, Medical Faculty, University of Malaya, Malaysia. The MAb produced by this hybridoma detected the presence of 17-OHP in the samples of serum, to diagnose CAH. This novel hybridoma became the focus of this study for developing a bioreactor-based scalable and inexpensive process for producing the MAb for possible use in clinical testing.

**CULTURE PROCESS**

Animal cells can be grown in continuous culture or in batch operations. As a result of the absence of equipment such as media and product holding tanks, pumps, etc., the cost of operating a batch system of a similar working volume as a continuous system is generally much lower (Marquis et al., 1990). The complexity of continuous culture systems and their susceptibility to contamination and mechanical failure (Marquis et al., 1990; Bibila and Robinson, 1995; Tokashiki and Yokohama, 1997; Dalm et al., 2005) is a further disadvantage. Moreover, it is wasteful to discard the valuable serum and nutrient components in the effluent stream (Glacken et al., 1983). Fouling and blockage of the cell retention devices in perfusion culture are other problems that have not been satisfactorily solved (Woodside et al., 1998; Kretzmer, 2002; Voisard et al., 2003). Compared with other operation modes, batch culture has a lower risk of contamination and mechanical failure as it needs no ancillary equipment. Therefore, batch modes of operation will be the primary focus in this study.

Currently, a monoclonal antibody that is able to detect 17OHP is being produced in cyclic-batch culture using CL-6 and CL-1000 culture flask (Integra Bioscience, Switzerland). These are static cultures with no dissolved oxygen and pH control. Thus, the amount of MAb obtained is rather low. In order to prepare a substantial amount of this MAb for clinical trials, a large amount of MAb has to be produced in a short time. Scaling-up the culture using a fully-controlled stirred batch bioreactor appears to be the preferred production option. This option will be examined for the hybridoma 192.

**SERUM-FREE MEDIA**

Using RPMI 1640 as a basal medium with the presence of insulin, transferrin and non-essential amino acids, Chang et al. (1980) successfully developed a serum-free medium for their hybridoma cell line (NS-19). They reported that the specific antibody production rate of their hybridoma in this serum-free medium was comparable to that in the serum-containing medium, although the maximum cell density achieved was greatly reduced. In another study, Kovář and Franěk (1986) supplemented RPMI 1640 with insulin, transferrin, ethanolamine, ascorbic acid, hydrocortisone and trace elements in culturing PTF-02 and T3-03 cells. This recipe was sufficient to support cell growth and produce an amount of MAb that was comparable to the level obtained in the serum-containing medium.

Several other basal media have been used to develop serum-free formulations for
hybridoma growth. For example, Chua et al. (1994) used RPMI 1640, DMEM/F12 and eRDF supplemented with insulin, transferrin, ethanolamine, selenium and bovine serum albumin. The specific growth rates for the hybridoma cell line (2HG11) were lower in all three types of serum-free basal media tested as compared to their serum-supplemented counterparts. Nevertheless, MAb titer obtained in serum-free eRDF medium was the highest (~ 193 mg/L) among the media tested in static culture for about 7 days. Martial et al. (1995) employed IMDM/F12 (1:1) as the basal medium and supplemented it with L-glutamine, glucose, iron-saturated human transferrin, polyethylene glycol, ethanolamine, mercaptoethanol, ascorbic acid, sodium selenite, essential amino acids, nonessential amino acids, bovine insulin and liposomes. They obtained cell density and MAb titer (25 mg/L) that were comparable to the values seen in the serum-containing medium during continuous culture of VO 208. It appears that a long list of supplements is not necessary for improving cell density and Mab productivity, as clearly seen for the cases of Kovář and Franěk (1986) and Martial et al.(1995).

Proprietary serum-free media such as HB101 (Franco et al., 1999), HB GRO (deZengotita et al., 2002a and 2002b), ASF103 and ASF104 (Terada et al., 2002) have also been used to culture hybridoma cells for producing monoclonal antibodies. The maximum cell density and MAB titer (25 mg/L) that were comparable to the values seen in the serum-containing medium during continuous culture of VO 208. It appears that a long list of supplements is not necessary for improving cell density and Mab productivity, as clearly seen for the cases of Kovář and Franěk (1986) and Martial et al.(1995).

Factors affecting cell growth, cell metabolism and antibody production

Temperature

In order to simulate normal body environment, mammalian cell cultivations are typically run at 37 C. For culture in T-flasks, culture flasks and roller bottles, the temperature is controlled via heated air in a CO2 incubator. In bioreactors, temperature is normally maintained through a re-circulating water jacket around the vessel or by a hot air bath.

Generally, optimal growth temperature for mammalian cells is 33 to 38C. Several researchers attempted to optimize the culture process by varying the culture temperature (Reuveny et al., 1986b; Sureshkumar and Mutharasan, 1991; Borth et al., 1992; Bloemkolk et al., 1992; Barnabé and Butler, 1994; Hovey et al., 1994; Chuppa et al., 1997; Yoon et al., 2004; Clark et al., 2004). The temperature range studied ranged from 29 C to 42 C. However, no growth was observed at both 29 C and 42 C. Optimum growth at 33 C was reported for hybridoma cells (HB-32) in serum-supplemented static culture. A lower optimal growth temperature (31 C) was reported by Reuveny et al. (1986b) for their hybridoma VIII H-8 in serum-containing static culture. In contrast, Bloemkolk et al. (1992) observed optimum growth at 37 C for the static culture and 34 C for the agitated culture for hybridoma S4B6 in a serum-containing medium. On the other hand, Barnabé and Butler (1994) who cultured their hybridoma CC9C10 in a serum-free medium observed similar growth rates and maximum viable cell counts at 33 C and 37 C. All the reported studies above showed extended culture viability at lower temperature. Hence, it appears that different cell lines show different optimum growth temperature, which is limited to the range of 33 to 37 C. Reported an increased cell metabolism.
when the culture temperature was increased. The glucose and glutamine uptake rates, and lactate and ammonium consumption rates, increased as the temperature increased. Similar observations were reported by Barnabé and Butler (1994). Nevertheless, Barnabé and Butler (1994) reported a constant yield of lactate from glucose at all the temperatures studied found an increased lactate yield from glucose as the temperature increased. These differences in observations might be due to the different culture media used, serum-supplemented medium, but Barnabé and Butler (1994) used a serum-free medium.

Owing to the different optimum temperature for growth and MAb production, several researchers attempted to optimize the productivity by temperature shift. Cells were first cultured at the optimum temperature for growth in order to achieve comparable amount of cells. Temperature was then shifted to a value that was optimal for MAb production. In such a temperature shift study, Similarly, Barnabé and Butler (1994) and Reuveny et al. (1986b) found that no significant improvement in MAb yield were obtained with the temperature shift strategy.

One study reported that cells had better resistance to shear at lower temperature (Ludwig et al., 1992). Lower culture temperature was also shown to reduce proteolytic activity, which is detrimental to the product quality (Chuppa et al., 1997) and stability. A reduction in oxygen consumption rate following a decrease in culture temperature was also observed (Chuppa et al., 1997; Jorjani and Ozturk, 1999). This suggests that a relatively high cell density can be supported in an oxygen transfer limited bioreactor by reducing the culture temperature (Chuppa et al., 1997).

In summary, it appears that the response of a cell line to different culture temperatures is dependent on the type of cell line, the medium type and the culture conditions.

**CONCLUSION**

Scale up of hybridoma 192 culture from the 2 L bench scale bioreactor to the 20 L bioreactor was successfully achieved using the constant impeller tip speed as the scale up criterion. A specific growth rate of 0.401/h and a maximum viable cell density of 1.89 × 10^6 cells/mL were achieved in the 20 L bioreactor. These values were higher than those in the 2 L bioreactor. However, the maximum MAb titer in the 20 L bioreactor was 18% lower than in the 2 L bioreactor. After going through several modifications in the cells’ environment from static to stirred culture, the MAb produced by hybridoma 192 is still specific to its antigen, 17-OHP. In addition, the scale up process did not compromise the structural integrity of the MAb. Hybridoma 192 could be grown in a low-cost low-serum medium. The specific growth rate and the average specific MAb production rate in the new medium were comparable to data obtained in DMEM supplemented with 5% FBS, 1% antibiotic antimycotic and 4 mM L-glutamine. Fed-batch cultivation of cells in spinner flasks demonstrated that an improved oxygen supply could improve the specific growth rate relative to static cultures. In addition, use of the fed-batch operation enhanced the total maximum viable cells numbers attained and extended the culture lifespan. However, this was at the expense of the average specific MAb production rate. The growth rate of the hybridoma 192 was reduced when cultivated in a sparged and stirred bioreactor, possibly because of the shear effects, but the average specific MAb production rate was improved. This was because of an inverse relationship between
the growth rate and the MAb production rate.

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