Development of hybridoma cells

ASSIGN BUSTER

Hybridoma cells are made from fusion of myeloma cells with B-cell lymphocytes obtained from a spleen of immunized host, usually from mouse. Myeloma cells have immortality properties but do not produce antibodies whereas B-cell lymphocytes are antibodies producer but they have short life span. When both cells are fused together, both the properties merge to form hybridoma cell line which is both immortal and produces antibodies continuously. The antibodies produced are monoclonal antibodies and are highly specific to a certain antigen (G Köhler & Milstein, 1976; Georges Köhler & Milstein, 1975).

Upstream

A good candidate of tumour cell must not produce antibodies itself and the tumour cell must be capable to fuse with B-lymphocyte (Shulman, Wilde, & Köhler, 1978) Tumour cell which has lost ability to express immunoglobulin but is able to form hybridoma cell that can produce pure specific monoclonal antibodies One example is the sub-clone of the mouse myeloma cell line P3-X63-Ag8, clone X63-Ag8. 653 which does not express antibodies but possess the ability to form hybridoma by fusion with B-lymphocyte cell (Kearney *et al.* , 1979).

To select a suitable cell line for antibodies production, certain variations that exist between the cell lines must be examined. Some of the differences are productivity, production pattern, genetic stability and cellular properties.

A cell line which expresses high amount of endoplasmic reticulum is capable to produce higher amount of antibodies. Product formation pathway and

route of product secretion also play an important role in product quality and quantity (Al-Rubeai *et al.* , 1990).

A mutant cell line which is sensitive to Hypoxanthine Aminopterin Thymidine (HAT) medium is necessary to isolate hybridoma cells from unfused B-cell lymphocytes and tumour. Human-human hybridomas have more functional applications compared to mouse-human chimeric hybridoma because human-human hybridoma cell Example of a human tumour cell line that can be used is U-266 human melanoma cell line (Olsson & Kaplan, 1980)

Monoclonal antibody titre can be increased by transforming Epstein-Barr virus into cell line. By the viral transformation, the genetic stability of the cell line also can be improved (Kozbor *et al.* , 1982).

Production of antibodies by transfecting novel antibody gene constructed through recombinant DNA technology and transfecting it into suitable vector provides a new way to study the properties, function and structure of the antibody molecules (Morrison *et al.* , 1984).

Medium

A common medium used in most system is the RPMI 1640 (Olsson & Kaplan, 1980). Usually the medium is enriched with foetal bovine serum (Legazpi *et al.* , 2005) because high antibody production can be obtained via high concentration of serum in the medium (Ozturk & ØPalsson, 1990). However serum free media that can produce the same result as with serum rich is preferred due to ease of purification.

Product

Hybridoma cells are used to produce monoclonal antibodies specific to the antigen the B-cells are immunized against. The product is extracellular (Georges Köhler & Milstein, 1975).

Bioprocess strategy

For sp2/0 cell line, the optimum pH which produces the most viable cell concentration is in the range of 7. 1 and 7. 4. However, an extreme pH result in higher antibody production which may suggest that under environmental or nutritional stress improves antibody titre (W. M. Miller *et al.* , 1988).

The optimum Disolved oxygen (DO) for antibody production by sp2/0 cell line is 50%. However the cells grow best at 0. 5% DO. At lower than 0. 5% DO the cell concentration declined because of incomplete glutamine oxidation. (William M. Miller *et al.* , 1987)

Best yield of antibodies can be achieved at 37 °C (Bloemkolk *et al.* , 1992). Shear forces are dependent the cell line and the type of reactor system used.

Growth

Antibody production is non-growth associated production. A lower specific growth rate results in higher specific antibody production rate (W. M. Miller *et al* ., 1988) According to (Craig Seamans & Hu, 1990), for the cell line AFP-S7 in a continuous stirred tank reactor the specific growth rate, glucose consumption and antibody production reduces over time.

In a continuous system, viability of the cells is highly affected by the dilution rate especially close to the extreme dilution rate. The concentration of antibodies produced and productivity reach its peak when the dilution rate is highest (Ray *et al.* , 1989).

Mode

Batch

Production using batch mode is simple and easy to maintain. Contamination can be minimized (Feder, 1985).

Fed-batch

A fed-batch can easily increase the final product concentration over batch mode. Besides that, other advantages include maintain the operation simplicity while ensuring the quality comply with FDA regulation and the entire culture uses only the approved stock of medium. Lastly, problems such as genetic drift and contamination could be minimized (Tremblay *et al.* , 1992).

Continuous

Waste metabolites, such as ammonia and lactate, at a concentration of more than few mM can be toxic for the growth of cells. Hence, it is crucial to exchange the medium by a perfusion system (Tayo *et al.* , 1986).

The flow rate of the system needs to be optimized with the specific growth rate of the cells and a suitable mechanism that retains the cell in the medium must be included in the system. Example of such retainer

mechanisms are continuous centrifuges, tangential flow membrane filters, dynamic filters, spin-filters, ultrasonic and dielectrophoretic separators, gravity settlers, and hydrocyclones (Dong *et al.* , 2005).

An example of the continuous system recommended for the hybridoma culture is the immobilized cells on fixed bed. Cells can be immobilization by entrapment in particles such as polymer or beads to make the separation between the medium and the cells simple, and yet ensure high growth of the cells until it reaches optimum concentration (Shirai, Hashimoto, Yamaji, & Tokashiki, 1987). In the case of immobilized system, the oxygen supply within the macroporous carrier determines the metabolism of the cells (Pörtner *et al.* , 1997).

Parameter	Batch	Continuous
Cultivation time (h)	240	260
Working volume (L)	1	1
Spent medium (L)	1	23. 5
Max viable cell density, $^{max} X_V$ (10^5 cells/mL)	30	350
Max total cell density, $^{max} X_T$ (10^5 cells/mL)	55	690
Av specific growth rate, $^{av} {}^{e}\mu$ (h^{-1})	0.	0. 033

Parameter	Batch	Continuous
	032	
Max mAb concentration (mg/L)	146	1270
Total amount of product (g)	0. 146	8. 06
Av specific production rate, $^{ave}q_{mAb}$ (pg/cell·h)	0. 6	0. 80 (limited cell growth); 2. 15 (stationary)
Productivity (mg/L·day)	14. 6	690
mAb yield (mg/L medium)	146	340

Difference between hybridoma production in batch mode versus continuous. Source : Dong et al. (2005)

Downstream

Product recovery

One of the methods of product recovery is by the use of cation exchange process (Thömmes *et al.* , 1995). Besides that, the product can be recovered using high performance liquid chromatography (HPLC) system with three columns, Protein A affinity chromatography, hydroxyapatite chromatography and linear gradient elution and endotoxin removing-gel chromatography. This method is used for scale of up to 1 g per batch (Horenstein *et al.* , 2003).

Target of product

The application includes monoclonal antibodies for analysis, detection and health purposes (Feder, 1985).

References

Al-Rubeai, M., Mills, D., & Emery, A. N. (1990). Electron microscopy of hybridoma cells with special regard to monoclonal antibody production. *Cytotechnology, 4* (1), 13-28. doi: 10. 1007/BF00148807

Bloemkolk, Jan-Willem, Gray, Murray R., Merchant, Fahar, & Mosmann, Timothy R. (1992). Effect of temperature on hybridoma cell cycle and MAb production. *Biotechnology and Bioengineering, 40* (3), 427-431. doi: 10. 1002/bit. 260400312

Craig Seamans, T., & Hu, Wei-Shou. (1990). Kinetics of growth and antibody production by a hybridoma cell line in a perfusion culture. *Journal of Fermentation and Bioengineering, 70* (4), 241-245. doi: http://dx. doi. org/10. 1016/0922-338X(90)90056-3

Dong, Haodi, Tang, Ya-Jie, Ohashi, Ryo, & Hamel, Jean-François P. (2005). A Perfusion Culture System Using a Stirred Ceramic Membrane Reactor for Hyperproduction of IgG2a Monoclonal Antibody by Hybridoma Cells. *Biotechnology Progress, 21* (1), 140-147. doi: 10. 1021/bp049826l

Feder, Joseph. (1985). *Large-scale mammalian cell culture* : Elsevier.

Horenstein, Alberto L., Crivellin, Federico, Funaro, Ada, Said, Marcela, & Malavasi, Fabio. (2003). Design and scaleup of downstream processing of

monoclonal antibodies for cancer therapy: from research to clinical proof of principle. *Journal of Immunological Methods, 275* (1–2), 99-112. doi: http://dx. doi. org/10. 1016/S0022-1759(03)00006-1

Kearney, John F., Radbruch, Andreas, Liesegang, Bernhard, & Rajewsky, Klaus. (1979). A New Mouse Myeloma Cell Line that Has Lost Immunoglobulin Expression but Permits the Construction of Antibody-Secreting Hybrid Cell Lines. *The Journal of Immunology, 123* (4), 1548-1550.

Köhler, G, & Milstein, C. (1976). Derivation of specific antibodyâ€ producing tissue culture and tumor lines by cell fusion. *European journal of immunology, 6* (7), 511-519.

Köhler, Georges, & Milstein, César. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature, 256* (5517), 495-497.

Kozbor, Danuta, Lagarde, Alain E, & Roder, John C. (1982). Human hybridomas constructed with antigen-specific Epstein-Barr virus-transformed cell lines. *Proceedings of the National Academy of Sciences, 79* (21), 6651-6655.

Legazpi, Lorea, Díaz, Jaime, Laca, Adriana, & Díaz, Mario. (2005). Kinetic analysis of hybridoma cell culture in a protein-free medium: Substrate and agitation effects. *Biochemical Engineering Journal, 26* (2–3), 122-130. doi: http://dx. doi. org/10. 1016/j. bej. 2005. 04. 009

Miller, W. M., Blanch, H. W., & Wilke, C. R. (1988). A kinetic analysis of hybridoma growth and metabolism in batch and continuous suspension

culture: Effect of nutrient concentration, dilution rate, and pH. *Biotechnology and Bioengineering, 32* (8), 947-965. doi: 10. 1002/bit. 260320803

Miller, William M., Wilke, Charles R., & Blanch, Harvey W. (1987). Effects of dissolved oxygen concentration on hybridoma growth and metabolism in continuous culture. *Journal of Cellular Physiology, 132* (3), 524-530. doi: 10. 1002/jcp. 1041320315

Morrison, Sherie L, Johnson, M Jacqueline, Herzenberg, Leonard A, & Oi, Vernon T. (1984). Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proceedings of the National Academy of Sciences, 81* (21), 6851-6855.

Olsson, L, & Kaplan, H S. (1980). Human-human hybridomas producing monoclonal antibodies of predefined antigenic specificity. *Proceedings of the National Academy of Sciences, 77* (9), 5429-5431.

Ozturk, Sadettin S, & ØPalsson, Bernhard. (1990). Loss of antibody productivity during long-term cultivation of a hybridoma cell line in low serum and serum-free media. *Hybridoma, 9* (2), 167-175.

Pörtner, Ralf, Rössing, Sabine, Koop, Matthias, & Lüdemann, Ines. (1997). Kinetic studies on hybridoma cells immobilized in fixed bed reactors. *Biotechnology and Bioengineering, 55* (3), 535-541. doi: 10. 1002/(SICI)1097-0290(19970805)55: 3 <535:: AID-BIT10> 3. 0. CO; 2-F

Ray, N. G., Karkare, S. B., & Runstadler, P. W. (1989). Cultivation of hybridoma cells in continuous cultures: Kinetics of growth and product

formation. *Biotechnology and Bioengineering, 33* (6), 724-730. doi: 10. 1002/bit. 260330610

Shirai, Yoshihito, Hashimoto, Kenji, Yamaji, Hideki, & Tokashiki, Michiyuki. (1987). Continuous production of monoclonal antibody with immobilized hybridoma cells in an expanded bed fermentor. *Applied microbiology and biotechnology, 26* (6), 495-499.

Shulman, Marc, Wilde, CD, & Köhler, Georges. (1978). A better cell line for making hybridomas secreting specific antibodies.

Taya, Masahito, Mano, Takashi, & Kobayashi, Takeshi. (1986). Kinetic expression for human cell growth in a suspension culture system. *Journal of fermentation technology, 64* (4), 347-350.

Thömmes, Jörg, Halfar, Markus, Lenz, Suzanne, & Kula, Maria-Regina. (1995). Purification of monoclonal antibodies from whole hybridoma fermentation broth by fluidized bed adsorption. *Biotechnology and Bioengineering, 45* (3), 205-211. doi: 10. 1002/bit. 260450304

Tremblay, M., Perrier, M., Chavarie, C., & Archambault, J. (1992). Optimization of fed-batch culture of hybridoma cells using dynamic programming: single and multi feed cases. *Bioprocess Engineering, 7* (5), 229-234. doi: 10. 1007/BF00369551