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Monoclonal Antibody Production: Media Optimization for Enhancement the Cell Viability of Hybridoma Cell

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Abstract: Media optimization of RC1 hybridoma cell culture for monoclonal antibody production was carried out in T-Flask experiment. The three identified important variables to affect the cell viability were studied. By using Central Composite Design of Response Surface Methodology (STATISTICA v 6.1) has shown that cell viability was maiuly affected by glutamine, serum and $NaCO_3$ concentration, respectively. Among the 16 runs tested, Run 11 indicated the best viability of the cell (>80% for five days). The critical values were obtained at 13.5, 1.68 and 0.87% for serum, glutamine and $NaCO_3$, respectively. These data were very significant where the p-values obtained for glutamine, serum and $NaCO_3$ were 0.000069, 0.003968 and 0.342151 (R^2 = 0.95476 and R Adj. = 0.88691), respectively.

Key words: Monoclonal antibody, RC1 hybridoma cell, cell viability, media optimization, central composite design, response surface method

INTRODUCTION

Antibody is a protein, synthesized and secreted by B-lymphocytes (B-cell) that bind to antigens. Antibody is members of a family of molecules (the immunoglobulin that constitute the humeral branch of the immune system) and form approximately 20% of the plasma proteins in humans. Different populations of immunoglobulin are found on the surface of lymphocytes, in exocrine secretions and in extravascular fluids (Pharmacia Biotech, 2000).

Monoclonal Antibody (MAb) is antibody that binds ouly to a specific antigen that compatible to its binding site (Xiao *et al*., 2005). Thus, in MAb production, there is no need for further purification in order to get the desired antibody as has been done for polyclonal antibody. MAb has become indispensable tools in research, diagnostics and therapeutics. They have gradually replaced the polyclonal antibodies since hybridoma technology was introduced (Zola, 2000).

The growth rate of mammalian cells (hybridoma cells) containing the antibody varies depending on the cell type, medium composition including the growth factors and other enviroumental conditions such as dissolved oxygen, carbon dioxide levels, pH and ionic strength (Butler, 1996; Constantino *et al*., 1995; Jung *et al*., 1992; Lee *et al*., 1991). Moreover, it is understood that the specific growth rate (μ) in hybridoma cells cultures starts to decline from the maximum level at 20 h of cultivation and continuously doing so until the growth eventually ceases (Doyle and Griffiths, 1998). Many studies had been conducted to optimize those parameters in optimizing the production of MAb (Satoshi *et al*., 2005; Heilmann *et al*., 2005; Lorea *et al*., 2005; Guez *et al*., 2004; Tibor *et al*., 2004; Ralf and Thomas, 1996).

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The main problem in producing MAb is cell apoptosis. Media optimization provides a way to reduce the chance of apoptosis and at the same time increase the hybridoma cell viability (Pakkanen and Neutra, 1994; Stoll *et al*., 1996). In this study, we had tried to optimize the media components of the RC1 hybridoma cells cultures not only to enhance the cells viability but also improve the production of MAb, using the Central Composite Design (CCD) method.

MATERIALS AND METHODS

Design of Experiment (DOE)

Experiment was conducted at Animal Cell Engineering Laboratory of IIUM and was designed by Response Surface Methodology (RSM) using a STATISTICA Software (Statsoft, 2001). RSM is a set of techniques designed to find the best value of response.

Cell Line

RC1 Hybridoma cell, a monoclonal antibody (IgG)-secreting cell line was purchased from Japanese Cell Culture Stock and had been used in this study.

Media Preparation and Optimization

RPMI media in liquid form was used for this optimization process. Formulation of media was first checked in order to determine the other component which is required. The RPMI media without L-glutamine was used and the three other components were added. The media was then taken into hood with any other supplement or addition that was required. The bottle was swabbed with 70% alcohol before uncap. For media optimization, 10 mL medium was prepared for 10 cm^2 T-flask used. Design of experiment was first done using STATISTICA to get the simulated value required for each variable of the 16 Runs.

The percentage of serum, sodium bicarbonate and L-glutamine needed to be added into each Run was designed as in Table 1. They were added according to the percentage volume of a total volume. Serum was added after the pH of media was adjusted to 7.2 and the media was then filtered.

Maintenance of Established Cell

The culture was examined carefully for any signs of contamination or deterioration. The cell was suspended carefully to homogenize the cell suspension. Five to seven milliliters medium was then

Table 1: Design of experiments of serum, sodium bicarbonate and L-glutamine

Run	DOE value for serum	Serum (%)	DOE value for $NaHCO_3$	$NaHCO_3$ (%)	DOE value for L-glutamine (%)	L-glutamine (%)
1	-1	5	-1	0	1	2
2	-1	5	-1	0	-1	0
3	1	15	-1	0	1	2
4	1	15	-1	0	-1	0
5	-1	5	1	2	1	2
6	-1	5	1	2	-1	0
7	1	15	1	2	1	2
8	1	15	1	2	-1	0
9	1	15	0	1	1	2
10	1	15	0	1	-1	0
11	0	10	0	1	0	1
12	0	10	1	2	0	1
13	-1	5	0	1	1	2
14	-1	5	0	1	-1	0
15	0	10	-1	0	0	1
16	0	10	-1	0	0	1

removed and discarded according to the need. Fresh medium was added up to 10-20 mL. The cell was dispersed into a single cell suspension by repeated pipetting. Volume was then maintained or split into two flask. The flask was then capped and stored in CO_2 incubator.

Cell Cultivation

All equipment such as 10 cm^2 T-flask, pipette, required media and inoculum were taken into hood. Nine milliliters media was transferred into a labeled T-flask using pipette. One milliliter of the inoculum was then taken and transferred into the T-flask making the volume in the T-flask became 10 mL. The fresh media and the inoculum were suspended in order to mix them well and then were incubated in 5% CO_2 incubator at 37°C. The cells were counted and their viability was determined by the trypan blue dye exclusion test. The counting result was then recorded for reference purpose.

Sampling

T-flask was put directly in hood from CO_2 incubator. Cap of T-flask was removed and it was held in the same hand that holds T-flask. Inoculum in T-flask was suspended aseptically in hood for 1-2 min to homogenize the hybridoma cell. Then, about 3 mL of cell was taken out using pipette into the centrifuge tube. About 10 μL of the cell in centrifuge tube was taken for cell counting. The rest of the cell in the centrifuge tube was centrifuged at 1000 rpm, 27°C for 10 min. The supernatant was collected for biochemical analysis.

RESULTS AND DISCUSSION

Media Optimization Result

All readings of the cell viability and the total cell number taken from the 16 T-flask on day 4 were entered into the STATISTICA software because most of the runs reach the highest cell viability in day 4 (data not shown). All measured readings were first transferred into based 10 values as shown in Table 2. The screen parameter is in percentage value.

From Analysis of Variance (ANOVA), it can be said that L-glutamine and serum were the main factors affecting the growth of hybridoma cell. The result was very significant since the p-value obtained for serum and L-glutamine was very small (0.003964 and 0.000069, respectively). From these values, it can be concluded that L-glutamine was a main factor that increased the viability of the hybridoma cell, followed by the serum (Table 3).

Table 2: Result from T-flask optimization

Run	Serum (%)	$NaHCO_3$ (%)	L-glutamine (%)	Log 10 (viability)	Log 10 (TCN)
1	5	0	2	5.43	6.08
2	5	0	0	4.60	5.15
3	15	0	2	5.83	6.18
4	15	0	0	5.05	5.61
5	5	2	2	5.59	6.01
6	5	2	0	4.81	5.18
7	15	2	2	5.79	6.19
8	15	2	0	5.31	5.77
9	15	1	2	5.67	6.20
10	15	1	0	5.21	5.73
11	10	1	1	5.65	6.06
12	10	2	1	5.51	6.08
13	5	1	2	5.69	6.08
14	5	1	1	5.39	5.35
15	10	0	1	5.65	6.00
16	10	0	1	5.65	6.15

Table 3: Critical value among the growth factors

	Critical values; variable: viability solution: maximum predicted value at solution: 5.82		
Factor	Observed minimum	Critical values	Observed maximum
Serum	5.00	13.50	15.00
$NaHCO_3$	0.00	0.87	2.00
L-Glutamine	0.00	1.68	2.00

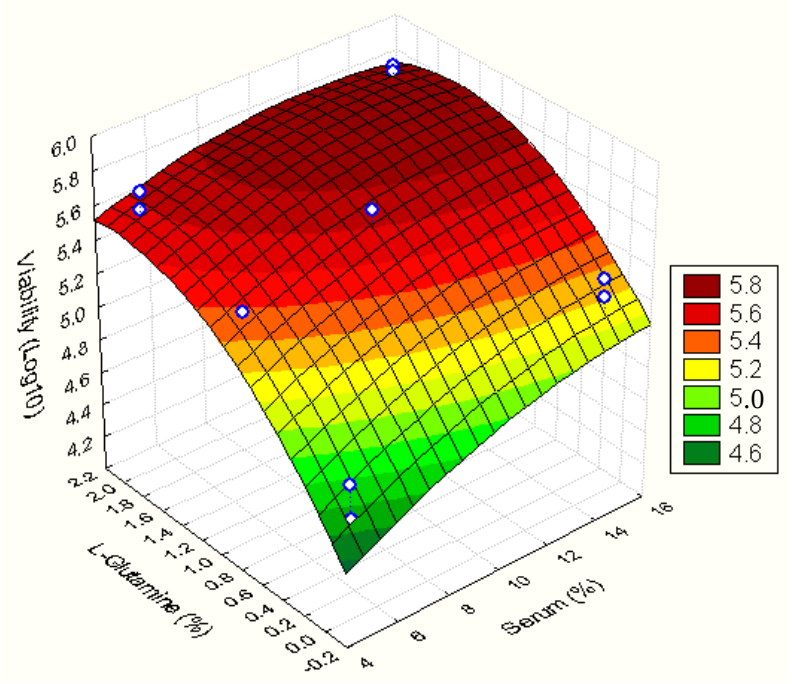

Fig. 1: Correlation between L-glutamine and serum on cell viability

Thus, the interaction between serum and L-glutamine in affecting the viability needs further clarification. It can be said that cell viability will increase as the percentage of both serum and L-glutamine increased. However, L-glutamine had influenced more than serum in increasing the cell viability. For example, when 14% serum was added in the RPMI media, cell viability would only increase when L-glutamine was added. Thus, the more serum added into the media, the more L-glutamine needs to be added in order to get higher cell viability (Fig. 1).

As shown in Fig. 2, cell viability increased as the percentage of serum increased. However, the addition of more $NaHCO_3$ does not affect the cell viability. For example, when adding 8% serum into RPMI media, the increase in the percentage of $NaHCO_3$ wonld only slightly increase the cell viability. This indicates that serum plays more important role to boost cell viability compared to $NaHCO_3$.

In Fig. 3, it can be observed that the cell viability increased as the percentage of L-glutamine increased. The $NaHCO_3$, however, does not affect the cell viability. For example when 1% of sodium bicarbonate was added into the RPMI media, cell viability has increased as the percentage of L-glutamine increased. However, when 1% L-glutamine was added into the media, there was no effect on cell viability even though there was an increasing in the percentage of $NaHCO_3$. The influence of L-glutamine on the cell viability was superior compared to that of the $NaHCO_3$.

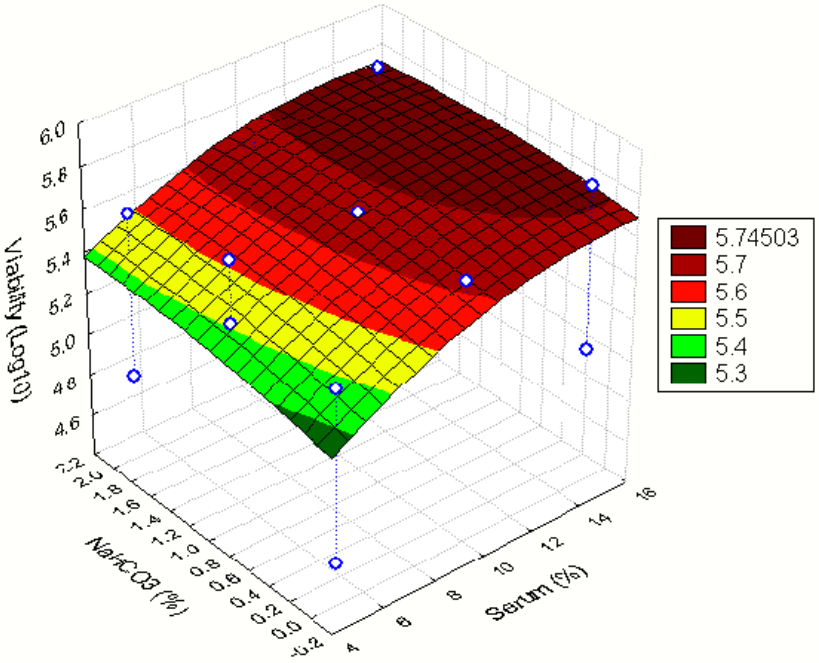

Fig. 2: Correlation between $NaHCO_3$ and serum on cell viability

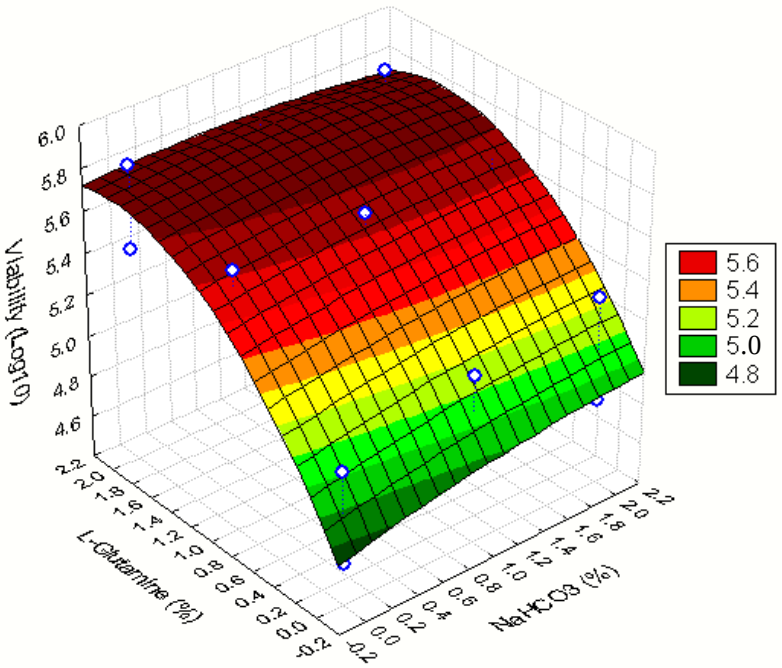

Fig. 3: Correlation between $NaHCO_3$ and L-glutamine on cell viability

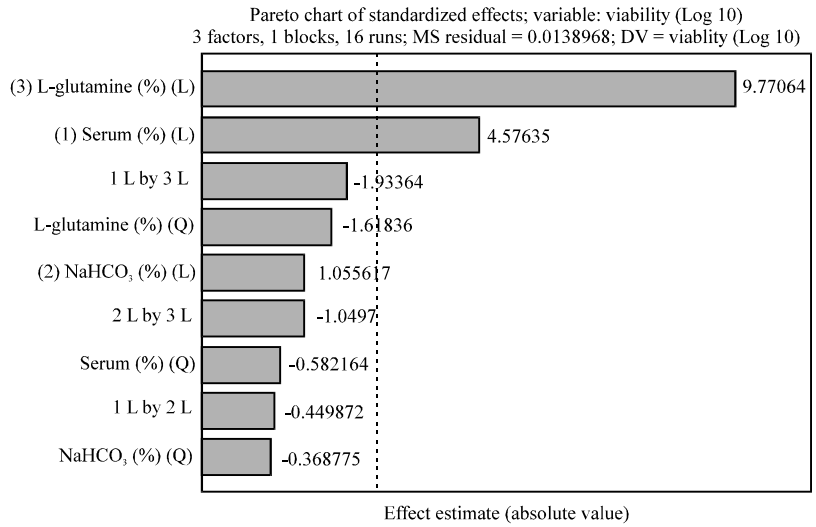

Fig. 4: Pareto chart of relatively importance correlation between independent screened parameters

Alternatively, STATISTICA had also provided the relatively importance correlation between the independent screened parameters in this research, which were serum, L-glutamine and $NaHCO_3$ by the Pareto chart (Fig. 4). This chart identified the most significant parameter to the dependent variable, in the case of this study, cell viability. The chart had also clearly showed that the L-glutamine was the most important component that influenced the hybridoma cell viability followed by serum and then combination between glutamine and serum.

At this point, it can be said that the main component that contribute most to the high hybridoma cell viability was L-glutamine. It has the largest effect (shown by p-value less than 0.05) on the increment of hybridoma cell viability.

Optimized Value of Serum, $NaHCO_3$ and L-glutamine

From the measured value of viability that was entered, STATISTICA gave critical value which is also called optimized value in percentage of serum, L-glutamine and $NaHCO_3$ (Fig. 4). It can clearly be seen that high percentage of serum was needed to get high cell viability where 13.5% serum was required compared to the need of only 1.68% of L-glutamine and 0.87% of $NaHCO_3$. This optimized value then can be used for the inoculation of the cell in a bigger bioreactor.

CONCLUSION

While many commercially available cell culture media exist, none are able to meet the specific requirements of every cell line. Optimization provides a way to increase the hybridoma cell viability which at the same time increases the production of MAb. Moreover, the addition of media component such as serum, L-glutamine and $NaHCO_3$ must be at sufficient amount in order to reduce the production cost.

REFERENCES

Butler, M., 1996. Animal Cell Culture and Technology: The Basics. IRL Press, New York.

Constantino, D., D. Pierre, F. Claude, L. Philippe and S. Marc, 1995. Adaptive predictive control of dissolved oxygen concentration in a laboratory-scale bioreactor. J. Biotechnol., 43 (1): 21-32.

Doyle, A. and J.B. Griffiths, 1998. Cell and Tissue Culture: Laboratory Procedures in Biotechnology. John Wiley and Sons Inc.

Guez, J.S., J. Ph. Cassar, F. Wartelle, P. Dhulster and H. Suhr, 2004. Real time in situ microscopy for animal cell-concentration monitoring during high density culture in bioreactor. J. Biotechnol., 111 (3): 335-343.

Heilmann, K., T.H. Groth, O. Behrsing, W. Albrecht, M. Schossig, A. Lendlein and B. Micheel, 2005. The influence of the chemical composition of cell culture material on the growth and antibody production of hybridoma cells. J. Biotechnol., 115 (3): 291-301.

Jung, H.C., D.K. Oh and J.H. Kim, 1992. Control of dissolved oxygen concentration in mammalian cell culture using a computer coupled mass flow controller. Biotechnol. Tech., 6: 405-408.

Lee, S.C., Y.B. Hwang, H.N. Chang and Y.K. Chang, 1991. Adaptive control of dissolved oxygen concentration in a bioreactor. Biotechnol. Bioeng., 37: 597-607.

Lorea, L., D. Jaime, L. Adriana and D. Mario, 2005. Kinetic analysis of hybridoma cell culture in a protein-free medium: Substrate and agitation effects. Biochem. Eng. J., 26 (2-3): 122-130.

Pakkanen, R. and M. Neutra, 1994. Bovine colostrum ultrafiltrate: An effective supplement for the culture of mouse-mouse hybridoma cells. J. Immunol. Methods, 169: 63-71.

Pharmacia Biotech, 2000. Handbook: Antibody Purification. Amersham AG.

Ralf, P. and S. Thomas, 1996. Modelling hybridoma cell growth and metabolism: A comparison of selected models and data. J. Biotechnol., 49 (1-3): 119-135.

Satoshi, T., M. Sasaki, K. Yanagihara and H. Yamada, 2005. Preparation of silk protein sericin as mitogenic factor for better mammalian cell culture. J. Biosci. Bioeng., 100 (6): 667-671.

Statsoft, 2001. STATISTICA for Windows (Volume IV): Industrial Statistics. Tulsa OK.

Stoll, T.S., K. Mühlethaler, U. Von Stockar and I.W. Marison, 1996. Systematic improvement of a chemically-defined protein-free medium for hybridoma growth and monoclonal antibody production. J. Biotechnol., 45 (I 2): 111-123.

Tibor, A., Z. Werner, P. Manfred and B. Jochen, 2004. On-line respiration activity measurement (OTR, CTR, RQ) in shake flasks. Biochem. Eng. J., 17 (3): 187-194.

Xiao, F.Y., H.L. Lin, S. Ming, L.L. Xiao, G. Jie, H.L. Yan and C.H. Zhong, 2005. Production of a monoclonal antibody against SARS-CoV spike protein with single intrasplenic immunization of plasmid DNA. Immunol. Lett., 100 (2): 177-181.

Zola, H., 2000. Monoclonal Antibodies: The Basics From Background to Bench. BIOS Scientific Publishers.