COMPARISON OF GROWTH RATE AND VIABILITY OF DF-1 CELL LINE IN DIFFERENT CULTURE MEDIA

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Abstract. *One of the commercial cell line that gained attention for the study of viral vaccine was an immortalized cell line derived from chicken embryo fibroblast (CEF) cells. Unlike CEF cells, DF-1 cells have higher proliferation rate and also can be subcultured infinite number of times. In this study, the growth profile of DF-1 cell line was investigated by undergoing series of optimization process by testing it out on several culture media and also by manipulating the components of culture media which were sodium bicarbonate and serum. At the end of this study it has been figured out that DF-1 achieved the highest cell culture density in Dulbecco's Modification of Eagle's Medium(DMEM) and with 1.24 g/L of $NaHCO_3$ and 5.5 % serum, it yielded the shortest doubling time.*

Keywords: cell culture, DF-1, optimization

1. Introduction

Animal cell culture is the most important tool in the study of animal cell structure, function and differentiation. It is also important for the production of many biologicals such as vaccines, enzymes, hormones, antibodies, interferons and nucleic acids. Most animal cells are anchorage-dependent and require attachment to a surface for their survival and replication (Adams, 1980). To improve the viability of any cell line for a targeted product formation, careful selection of an optimized media as well as other growth parameters are inevitable. Normally, the viability of a cell line increases upon the increase of duration time, but accumulation of toxic metabolic products such as lactate and ammonium, viable cell concentration drops after the stationary phase (Mel *et al; 2008*). Doubling time is an essential parameter in cell viability. Generally, the doubling time of mammalian cells varies between 10 and 50 hours, and cell concentration reaches its peak value within 3-5 days.

DF-1, named after the founder, Douglas Foster is a spontaneously immortalized continuous cell line of chicken embryo fibroblast (CEF). The cell line is derived from 10 day old East Lansing Line (ELL-0) eggs. The cells are useful as substrates for virus propagation, recombinant protein expression and recombinant virus production. DF-1 cell line has a high rate of proliferation under normal culture conditions. Kim *et al.,* (2001) reported that the rapidly dividing cell characteristic of DF-1 is contributed by three mitochondrial-encoded genes which are ATPase 8/6, 16S rRNA, and cytochrome b. These genes which are associated with mitochondrial respiratory functions are expressed at higher levels in DF-1 cells compared to primary and other immortal CEF cells. Increased mitochondrial respiratory functions give DF-1 higher rate of cell proliferation.

The objective of this study was to compare the growth rate and viability of DF-1 cell line in four different types of culture media – DMEM, DMEM/F12, MEM and RPMI 1640.

2. Materials and Methods

2.1. Cell lines

Cell line of DF-1 used in this study was bought from the American Type Culture Collection (ATCC). Cryovial was swirled in water bath at a temperature of 37^{o}C. The content was immediately transferred into a centrifuge tube containing 10 ml of media. This was centrifuged at 1000 rpm for 15minutes at 25^{o}C. Supernatant was discarded and 10 ml of fresh media was added to resuspend the pellet to form the inoculums stock.

2.2. Culture media

Dulbecco's Modification of Eagle's Medium, DMEM (with glucose and L-glutamine), Dulbecco's Modification of Eagle's Medium/F-12 1:1, DMEM-F12 (with glucose and L-glutamine), Roswell Park Memorial Institute medium (RPMI 1640), and Minimum Essential Medium (MEM) from Gibco® and fetal bovine serum (FBS) were used to culture and maintain the cells.

2.3. Media optimization

There were two stages for the optimization process. On the first stage, the cells were cultured using four different basal media which are Dulbecco's Modification of Eagle's Medium, DMEM (with glucose and L-glutamine), Dulbecco's Modification of Eagle's Medium/F-12 1:1, DMEM-F12 (with glucose and L-glutamine), Roswell Park Memorial Institute medium (RPMI 1640), and Minimum Essential Medium (MEM).

Later, after the basal media which yield the maximum viable cell number has been identified, the media was taken to the second stage. The second stage was performed according to the Design of Experiment with 3 Level of Factors generated by STATISTICA® software (Table 3.1). Two factors (independent variables) that were varied during the media optimization were the concentration of sodium bicarbonate, $NaHCO_3$ (0.5, 1.0, and 1.5 g/L) and the percentage of serum in the media (4, 7, and 10%).

2.4. Cell inoculation

Throughout this study, all procedures were carried out aseptically in a biohazard safety hood. To 12 labeled $25cm^2$ T-flasks for each of the 4 media, 9mls of fresh media was added and 1ml (1.7×10^4cells/ml) of the innocula was inoculated in each of the flasks and incubated in a CO_2 incubator at 37^oC with 5% CO_2 after proper mixing.

2.5. Sampling

A flask from each of the media was trypsinized and viable cell number was determined by counting using the hemocytometer as described by (Freshney, 2000). This was done after every 6hours for 3 days.

2.6. Trypsinization

In a safety biohazard hood, media from T-flask was discarded, washed twice with phosphate buffered saline (PBS), added 1ml trypsin and incubated in a CO_2 incubator for 5mins. Following cell detachment (by examination under microscope), 4ml fresh media was added and cells were homogenized by gentle flushing.

2.7. Cell counting

On a clean slide, 10µl of trypsinized cell suspension was placed and another 10µl of trypan blue was added to the suspension using a micropipette. This was mixed very well. On the prepared hemocytometer chamber, 10µl was placed at the edge of the cover slip and hemocytometer at one end and another 10µl at the other end. This was evenly distributed by capillary action. Only cells in the $1mm^2$ were counted. Cells in the four large squares of the hemocytometer were counted and divided by four to get the average value.

Table 1. Experimental design for optimization of culture media

Standard run	Parameter			
	$NaHCO_3$ concentration		Percentage of serum	
	Code	Actual (g/L)	Code	Actual (%)
1	-1	0.5	-1	4
2	-1	0.5	0	7
3	-1	0.5	1	10
4	0	1.0	-1	4
5	0	1.0	0	7
6	0	1.0	1	10
7	+1	1.5	-1	4
8	+1	1.5	0	7
9	+1	1.5	1	10

3. Results and Discussion

In the first part of the optimization process, in which DF-1 cells were cultivated in four different basal media, the maximum viable cell concentration was achieved by cultivation of cells in Dulbecco's Modification of Eagle's Medium or DMEM. The maximum viable cell concentration achieved was 1.24×10^6 cells/ml. The value is very high when compared to the second maximum viable cell concentration which is 8.10×10^5 cells/ml achieved by the cultivation in Roswell Park Memorial Institute medium or RPMI 1640. According to Hayman and Ruoslahti (1979), DMEM are able to supports the growth of cells to much higher densities compared to many other basal media because the concentrations of certain essential amino acids and vitamins are several fold greater in DMEM

than in the other media. From the growth kinetics aspect, Dulbecco's Modification of Eagle's Medium/F-12 1:1 or DMEM-F12 achieved the shortest doubling time which is 13.22 hours followed by RPMI 1640 (15.30 hours), DMEM (21.39 hours) and MEM (33.64hours). However, according to Table 3, the price of DMEM/F-12 is more expensive when compared to the other three media. High cost is usually avoided as it will become a major constraint when an experiment is scaled up to higher production level. Therefore DMEM is selected to be carried forward to the further stages of the study since it yield the maximum viable cell concentration and have relatively lower price. The selection of DMEM is also in line with the goals of an ideal optimization process which is to maximize the yield/profits and at the same time minimize the costs.

Table 2. Growth profile of DF-1 cells in four different basal media

Standard run	**Basal media**	**Maximum viable cell concentration (cells/ml)**	**Specific growth rate, μ (h^{-1})**	**Doubling time, t_D ($t_D = ln2/\mu$) (h)**
1	DMEM/F-12	7.65×10^5	0.0524	13.22
2	MEM	0.55×10^5	0.0206	33.64
3	RPMI 1640	8.10×10^5	0.0453	15.30
4	DMEM	1.24×10^6	0.0324	21.39

Table 3. Price list of basal medias (Gibco)

Basal media	**Size**	**Price (USD)**
DMEM/F-12	10 packets × 1L	45.10
MEM	10 packets × 1L	33.20
RPMI 1640	10 packets × 1L	32.30
DMEM	10 packets × 1L	34.70

In the second stage of the optimization process, the maximum viable cell concentration and the shortest doubling time were achieved during Run 8 where the concentration of $NaHCO_3$ and serum were 1.5 g/l and 7%. The maximum viable cell concentration achieved was 1.25×10^6 cells/ml and the doubling time analyzed was 16.62 hours. However, from the analysis produced by the STATISTICA®, the software deduced that the critical values that able to yield the shortest doubling time are 1.24 g/L of $NaHCO_3$ and 5.5 % serum in the culture medium.

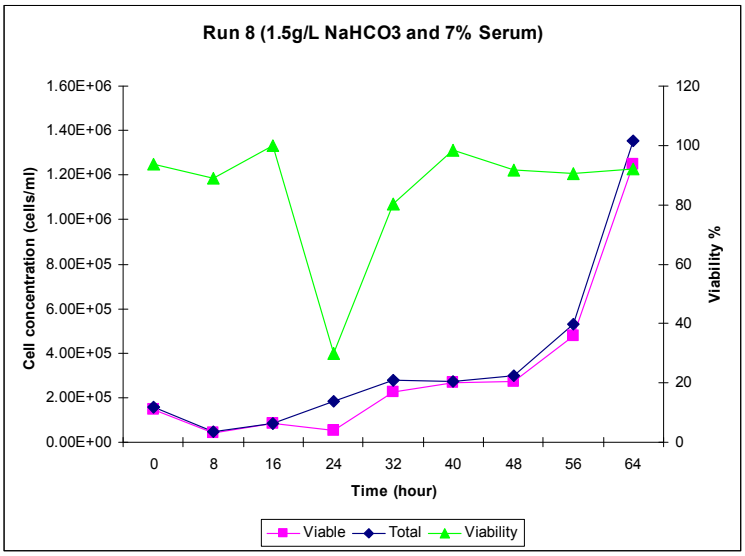

Figure 1. The growth profile of DF-1 cells in Run 8 (1.5g/L $NaHCO_3$ and 7% serum)

Table 3. Growth profile of DF-1 cells in different concentrations of serum and $NaHCO_3$

Standard run	$NaHCO_3$ concentration (g/L)	Percentage of serum (%)	Maximum viable cell concentration (cells/ml)	Specific growth rate, μ (h^{-1})	Doubling time, t_D ($t_D = \ln 2/\mu$) (h)
1	0.5	4	7.25×10^5	0.038	18.24
2	0.5	7	7.40×10^5	0.0255	27.18
3	0.5	10	3.45×10^5	0.0163	42.52
4	1.0	4	6.15×10^5	0.0317	21.87
5	1.0	7	6.90×10^5	0.027	25.67
6	1.0	10	6.15×10^5	0.0347	19.98
7	1.5	4	6.70×10^5	0.0329	21.07
8	1.5	7	1.25×10^6	0.0417	16.62
9	1.5	10	1.24×10^6	0.0324	21.39

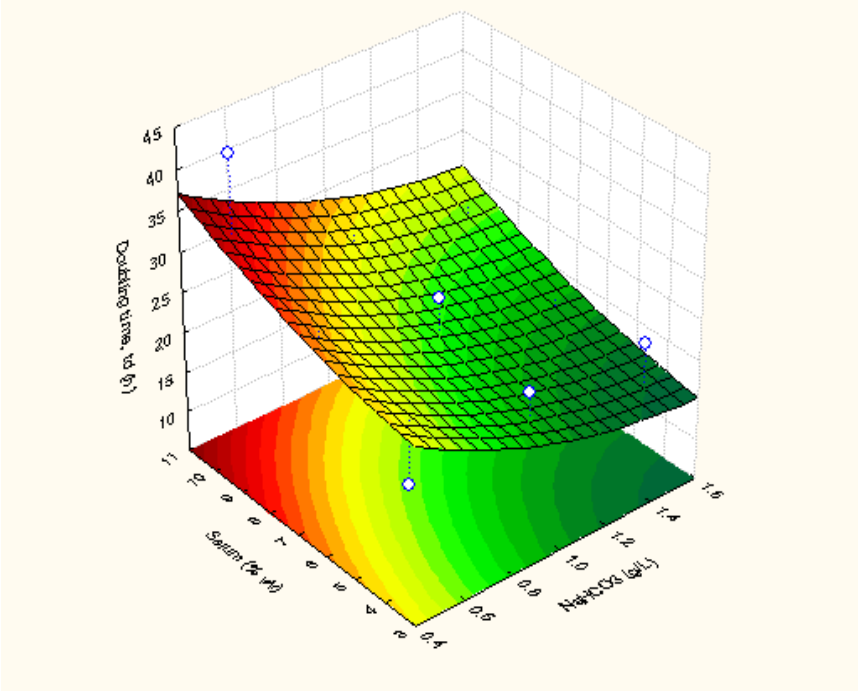

Figure 2. Effect of media composition on doubling time of DF1 cell

According to McClenaghan (1999), the function of $NaHCO_3$ in culture medium is to provide a buffer system to the pH of the culture medium. But the capacity of $NaHCO_3$ is often not sufficient to be able to prevent a decreasing pH towards the end of the culture cycle.

It is a different thing when it comes to serum concentration in the culture medium. Serum has a vital roles in the growth of cells. Serum provides the basic nutrients for the cells, growth factors and hormones which are involved in growth promotion and specialized cell functions, supplies proteins like fibronectin which promote attachment of cells to the substrate and binding proteins like albumin and transferrin which carry other molecules into the cell, increase viscosity of the medium thereby protects cells from mechanical damages, e.g., shear forces during agitation of suspension cultures, protect cells from proteolysis, provides minerals like Na^+, K^+, Fe_2^+, Zn_2^+, etc., and also act as buffer.

Nevertheless, despite of many vital roles carried by serum, the price of the supplement is very expensive. Reduction in the usage and dependant on serum in cell culture is well desired as it will decrease the cost of the experiment and reduce the restrictions in terms of cost when scaling up. The critical value of serum concentration

deduced by STATISTICA® based on the results of experiments was 5.5 % (see Figure 2), which is considered to be relatively low and satisfactory.

4. Conclusion

The growth profile of DF-1 cells in different culture media formulations has been determined. Among the common basal media used in cell culture, the one that suits best the DF-1 cell lines is Dulbecco's Modification of Eagle's Medium or DMEM. The extra nutrients available in DMEM compared to others allowed the cell population to grow to high density culture. While in the second stage, it has been figured out that the optimum concentration of $NaHCO_3$ and serum which able to yield the shortest doubling time were 1.24 g/L and 5.5 %. This step is important as it help to determine the optimum usage of $NaHCO_3$ and serum which are very pricey.

Acknowledgement

The authors would like to thank Ministry of Science Technology and Innovation (MOSTI) of Malaysia for funding this research under Project No. ABI (A) - 21.

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