# GROWTH STUDY OF DF-1 CELL LINE IN MICROCARRIER BIOREACTOR

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**Abstract.** *Animal cell culture is fundamental in the manufacturing of many biological products. Biological products produced in animal cell cultures include vaccines, enzymes, hormones, antibodies and anticancer agents. However majority of animal cells is anchorage-dependent and requires attachment to a surface for their growth. Microcarrier is a small bead used to provide surface for the culture of anchorage-dependent animal cells. Microcarrier culture has very large surface area to volume ratio, thus able to produce larger number of cells when compared to conventional monolayer cultures which uses multiple glass or plastic bottles. This research was carried out mainly to study the growth performance of DF-1 cell culture by using microcarriers in a 1-L litre stirred tank bioreactor and compares it with conventional monolayer cultures grown in T-flasks bioreactor. At the end of the study it has been found that the maximum viable cell concentration achieved by the microcarrier culture was 1.315 × $10^6$ cells/ml which was higher than maximum viable cell concentration achieved by the cell culture in T-flasks which was 1.25 × $10^6$ cells/ml.*

Keywords: DF-1, microcarrier, T-flask and bioreactor

## 1. Introduction

Animal cell culture is the most important tool in the study of animal cell structure, function and differentiation. Animal cell culture is also important for the production of many important biological materials such as vaccines, enzymes, hormones, antibodies, interferons and nucleic acids. The majority of animal cells is anchorage-dependent and requires attachment to a surface for their survival and replication (Grinnell *et al*., 1978; Adams, 1980; and Groot, 1995).

For large-scale production of animal cells an extensive surface is necessary for growth. Previously, the most popular methods for providing this surface involved multiple glass or plastic bottles. The surfaces available for growth were only those of the inside of the bottles. Such systems are labor intensive and require both a large amount of space and specific equipment handling. Further disadvantages in the bottle technique are the variation that can arise between different bottles such as the pH and the increasing risk of contamination as the number of units to handle increases.

Therefore number of alternative schemes for growing large quantities of anchorage-dependent cells has been examined to overcome this problem. These include spiral film, plastic bags, packed or fluidized beds, hollow fiber beds, and microcarrier culture. Out of these schemes, microcarrier culture has been the most successful of the different approaches.

Reuveny (1990) defined microcarriers as small (100-250$\mu m$ diameter) beads or rods with a surface for attachment and growth of anchorage dependent cells. Microcarrier culture introduces new possibilities and makes possible the practical high yield culture of anchorage-dependent cells. In microcarrier culture cells grow as monolayers on the surface of small spheres which are usually suspended in culture medium by gentle stirring. By using microcarriers in simple suspension culture systems it is possible to achieve yields of several million cells per milliliter (Clark *et al.,* 1999).

The project was carried out mainly to investigate the growth performance of DF-1 cell culture by using microcarriers in a 1-L stirred tank bioreactor and compares it with conventional monolayer cultures grown in T-flasks. DF-1 was incorporated in the study because it is an immortal cell line and has rapid proliferation rate.

## 2. Materials and Methods

### 2.1. Cell lines

A spontaneously immortalized continuous cell line of chicken embryo fibroblast (CEF) DF-1 was used throughout the study. The cell line is bought from American Type Culture Technology (ATCC). DF-1 cell line is actually

derived from 10 day old East Lansing Line (ELL-0) eggs.

**2.2. Culture media**
Dulbecco's Modification of Eagle's Medium, DMEM (with glucose and L-glutamine) from Gibco® and 10% fetal bovine serum (FBS) were used to culture and maintain the cells.

**2.2. Microcarrier**
Cytodex 1 microcarrier from Amersham Biosciences was used to provide the surface for cell attachment in the stirred tank bioreactor culture.

**2.3. Microcarrier preparation**
Dry microcarriers are added to a suitably siliconized glass bottle and are swollen in $Ca^{2+}$, $Mg^{2+}$-free PBS (50-100 ml/g microcarriers) for at least 3 hours at room temperature with occasional gentle agitation. The hydration process can be accelerated by using a higher temperature e.g. 37°C. The supernatant is decanted and the microcarriers are washed once with gentle agitation for a few minutes in fresh $Ca^{2+}$, $Mg^{2+}$-free PBS (30-50 ml/g microcarriers). The PBS is discarded and replaced with fresh $Ca^{2+}$, $Mg^{2+}$-free PBS (30-50 ml/g microcarriers) and the microcarriers are sterilized by autoclaving with steam from purified water (115°C, 15 min., 15 psi).

Prior to use the sterilized microcarriers are allowed to settle, the supernatant is decanted and the microcarriers are rinsed in warm culture medium (20-50 ml/g microcarriers). This rinse reduces dilution of the culture medium by PBS trapped between and within the microcarriers (a step of particular importance when using small culture volumes or cells with low plating efficiencies). Then the microcarriers are allowed to settle, the supernatant is removed and the microcarriers are resuspended in a small volume of culture medium. It is not necessary to treat the microcarriers with serum or to have serum in the rinsing medium.

**2.4. Cell culture in T-flask**
Cells were cultured in T-flask according to the protocols described by Freshney (2000). Confluent DF-1 cell cultures in a T-75 flask were subcultured into twelve T-25 flasks, provided that each flask would contain the same amount of culture media and size of cell inoculums. Sampling was done every eight hours by performing cell counting in one T-flask each time to determine the cell concentration.

**2.5. Cell culture in bioreactor**
A total of three bioreactor experiments were conducted with different concentrations of microcarriers which were 1g/L, 3g/L, and 5g/L. Prior to use the bioreactor is sterilized by autoclaving. After the bioreactor is completely set-up and proven to be completely sterilized, the culture medium was inserted into the bioreactor vessel and followed by the prepared microcarrier and the cells. The inoculum size that was used is 10% meaning that 100 ml of DF-1 cell broth was added to 900 ml of culture medium. Sampling was done every eight hours to determine the cell concentration by performing cell counting. Operating conditions of the bioreactor was as follows:

Table 1. Operating conditions for bioreactor experiment

| Parameter | Value |
|---|---|
| pH | 7.0 |
| Temperature | 37ºC |
| Impeller speed | 70 rpm |
| pO2 | 30 % |

## 3. Results and Discussion

In T-flask culture the maximum viable cell umber achieved was $1.24 \times 10^6$ cells/ml which was after 64 hours of cell inoculation. As can be seen in Figure1, the cells were in the lag phase from 0h to 48h. After adapted to the environment and produced enough materials to mass proliferate, the cells entered the exponential or the log phase after 48 hours of inoculation and reached the maximum viable cell number at the end of the phase.

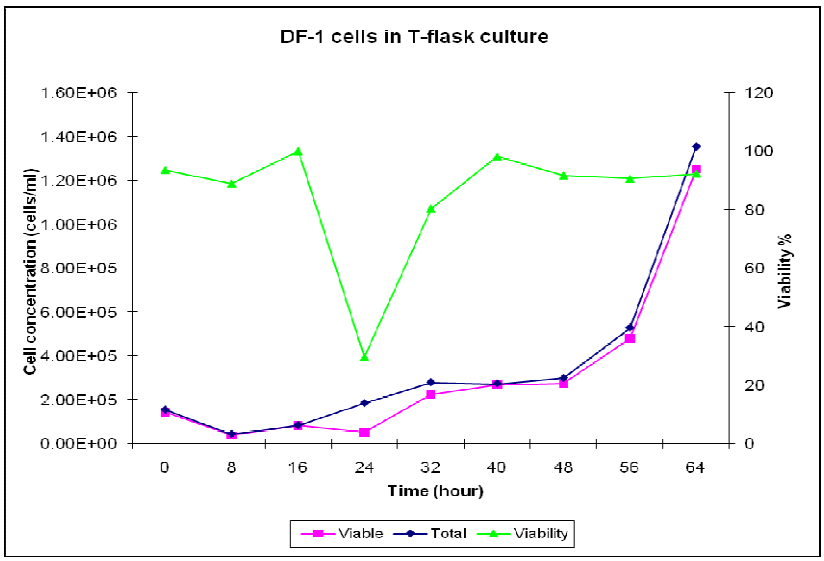
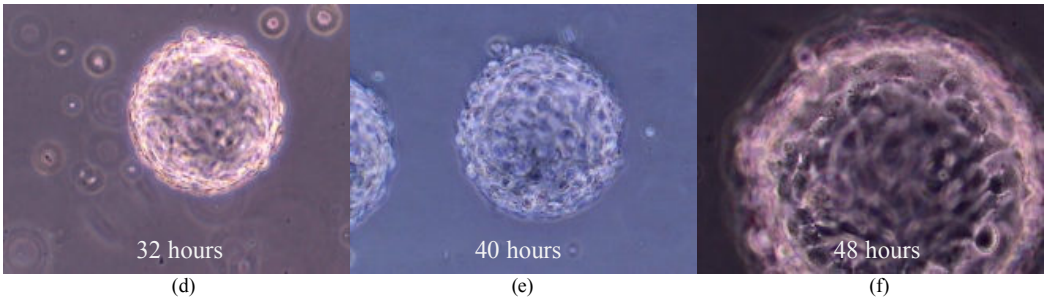



Figure 1. Growth profile of DF-1 cells in T-flask

Different combinations of microcarrier concentrations (1, 3 and 5 g/L Cytodex 1) were tested in a 1-litre stirred tank bioreactor (data not shown) to achieve high viable cell number. In the second run in which the concentration of microcarriers was 3g/L, maximum viable cell number has been obtained. Microscopic observation of DF-1 cells in the second run showed that cells randomly attached to the microcarriers after 8 hours of cell inoculation. Within 1 day nearly all of the inoculated cells had attached to the microcarriers and after 72 hours a maximum number of $1.315 \times 10^6$ cells/ml was reached. After reached the maximum, cells began to detach from the microcarriers and to form aggregates resulting in a significant drop in the number of viable cells, which is most likely due to medium limitations or an accumulation of inhibiting substances in the late phase of the cultivation.


(a) (b) (c)




(d) (e) (f)

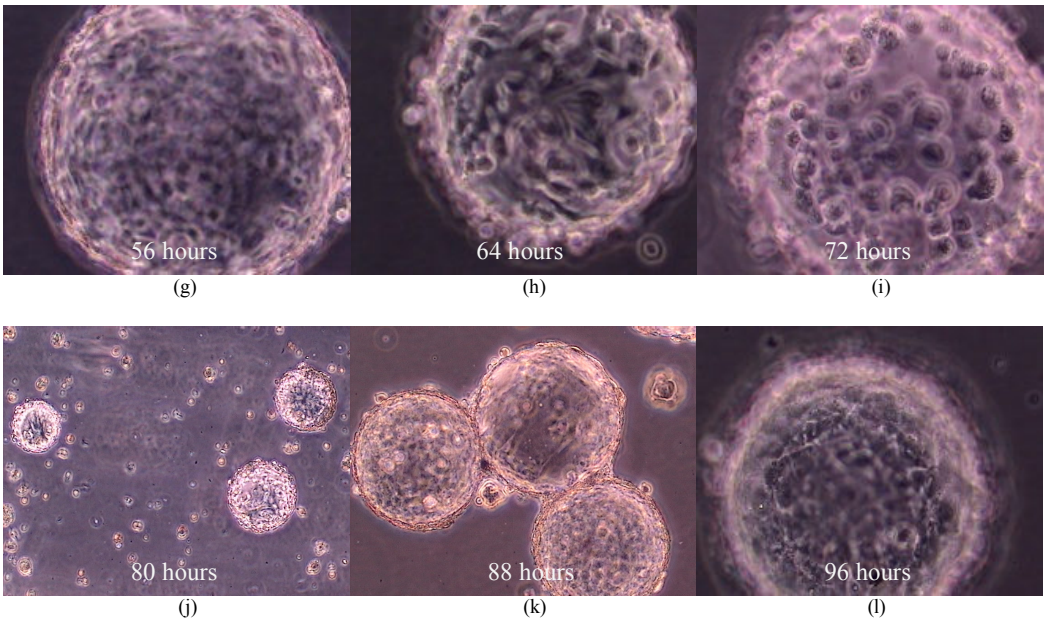



Figure 2. Microscopic pictures from DF-1 cells on Cytodex 1 microcarriers (a) – (l)

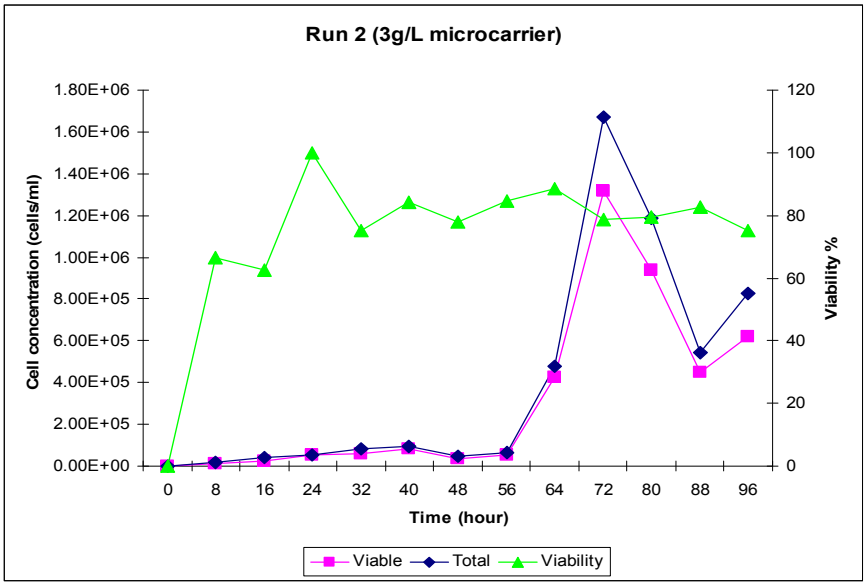



Figure 3. Growth profile of DF-1 cells in bioreactor (3.0g/L microcarrier)

As shown in Figure 3, the cell reach the highest viability (100%) at 24 hours with the total cell numbers about 2 x $10^5$ cells/ml, then the viability decreased to 80-90% until 96 hours of culture with the total cell numbers reached the maximum at 1.6 x $10^6$ cell/ml, then the cell number slowly decreased until at the end of the culture that was 96 hours. During this period many cells has been died or lysed because of dynamic force from bioreactor itself such as agitation and aeration and also accumulation of byproduct such as lactate and ammonia that will kill or destroy the cell. When it compared to shake flask culture, bioreactor was much better in terms of the controlling the conditions such as pH, temperature that can generate the high cell numbers in bioreactor. The most important in this study is to prolong cell viability in high percentage and to get the maximum cell numbers. At the end will be used for inoculating of virus for vaccine production.

## 4. Conclusion

The ability of microcarriers in producing higher cell density cultures has been proven by achieving maximum viable cell concentration of $1.315 \times 10^6$ cells/ml which is from the second run of the bioreactor experiment where the concentration of microcarrier was 3g/L. The achieved value is slightly higher than what was achieved in T-flask culture which was $1.25 \times 10^6$ cells/ml. Large surface area provided by the microcarrier beads allow more space for cells to attach and grow until reach the confluent state with cell density more than conventional monolayer culture.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