Bead-to-bead transfer of Vero cells in microcarrier culture

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Abstract

Cell harvesting technique is of considerable importance in the scale-up of microcarrier cultures of anchorage-dependent cells. The traditional methods are often time- and labor-consuming and cause physiological damage to the cells. Bead-to-bead cell transfer provides an attractive solution to the scale up process. By intermittent agitation, successful cell transfer was achieved. Significant cell growth was observed where bare beads contacted with confluent ones. Most of the fresh microcarriers reached near confluence four days after addition into the culture medium.

Introduction

Microcarrier technology has been applied successfully to the cultivation of anchorage-dependent cells and the production of many important biological materials, such as vaccines, tissue plasminogen activator, human β interferon, Erythropoietin, etc. However, only a few of them have been transposed to industrial scale because of the difficulties encountered in the scale-up process. And in most cases, cell subculturing techniques is one of limiting factors.

The most popular cell harvesting techniques are enzymatic ones which include proteolytic enzymes, e.g., trypsin and collagenase. Chelating agents (EDTA), hypotonic treatment, low temperature treatment and sonication, alone or in combination, can also be used to detach cells from the microcarriers (Pharmacia Fine Chemicals, 1982). Unfortunately, these methods are time and manpower draining and often do physiological to the cell membrane which leads to unfavorable cell growth in the following culture.

Bead-to-bead cell transfer provides an alternative solution to the scale-up of microcarrier cultures. By bead-to-bead cell transfer, an easy scale-up process can be obtained by addition of fresh microcarriers and fresh medium to the small confluent culture. The damage done by traditional harvesting methods becomes

negligible in this method. The success of bead-to-bead transfer depends on the adhesive property of the cells and the characteristics of the microcarrier surface (Deyu Kong, 1998). Only a few kinds of microcarriers and cells can meet the requirement. Researches now available are often carried out on recombinant Chinese Hamster Ovary cells growing on macroporous microcarriers (made of gelatin or cellulose) (Kiyoshi Kamiya, 1995; M. Butler, 1988; S. Ohlson, 1994).

In this study, bead-to-bead transfer of Vero cells was examined by addition of fresh Cytodex-3 microcarriers to a near-to-confluent culture in a spinner flask. The mechanisms and behavior of bead-to-bead cell transfer on new and recycled microcarriers were studied.

Materials and methods

Cell line and maintenance

Vero, the continuous African Green Monkey kidney cell line, was kindly provided by the National Serum and Vaccine Institute, Beijing, P. R. China. The cells were maintained in a T-75 flask with 25 mL culture medium (Medium 199 (Gibco BRL) supplemented with glucose (2.0 g/L) and 8% new-born bovine serum (Beijing, Dairy Co., P. R. China)) in 5% CO₂ incubator

at 37%. The cell passage was carried out at 80–90% confluence at 1:4 ~ 1:5 ratio with 0.15% trypsin in Ca^{2+} , Mg^{2+} free phosphate-buffered solution (PBS). The cells were counted using a haemocytometer before inoculation.

Microcarrier cell culture

Microcarrier cell culture was carried out in the 500-mL spinner flask containing a suspended magnetic impeller in a 5% CO_2 incubator at 37°C. Cytodex-3 microcarriers (Sigma Chemical Co., U.S.A.) were prepared as described (Pharmacia Fine Chemicals, 1982). The culture process was initiated by intermittent agitation (agitate for 5 min and keep static for 25 min) at about 60 rpm in the first 2 hr after inoculation, then the agitation speed was kept at 80 rpm. Samples were taken out every day for microscopic examination and growth determination. 50% of the culture medium was replaced daily two days after cell inoculation.

Preparation of recycled Cytodex-3 microcarriers

Cytodex-3 microcarriers with cells from a batch culture in a 5 L LH bioreactor were collected and washed twice with Ca^{2+} , Mg^{2+} free PBS (200 mL per gr microcarriers), fresh PBS with 0.01% EDTA (pH adjusted to 6.0 with 0.1 N NaOH) was then added, cooled to 4°C and agitated at 100 rpm for 24 hr, then fully rinsed with fresh PBS to remove the cell debris. The microcarriers so treated were then collected and prepared the same way as that for new microcarriers before use for another batch of cell culture.

Growth determination

1 mL of evenly suspended culture was taken out and allowed to settle for 15 min, the supernatant was discarded. The microcarriers with cells were washed with PBS once, then re-suspended in 0.1 M citric acid containing 0.1% (w/v) crystal violet and incubated at 37 °C for 1 hr. The mixture was then gently pipetted and the cell number was determined by counting the released nuclei with a haemocytometer.

Figure 1. Vero cell culture on recycled Cytodex-3 microcarriers.

Result and discussion

Bead-to-bead transfer of Vero cells grown on recycled Cytodex-3 microcarriers

Vero cells were cultured on about 0.4 g recycled Cytodex-3 microcarriers in 200 mL culture medium with an inoculation density of 1.5×10^5 cells/mL. 4 days later, the culture reached confluence (with a density of 9.0×10^5 cells/mL). Bare beads were then added at a ratio of 1:1 (bare : confluent) and the culture continued with an intermittent mode of agitation, i.e., agitated for hour and then kept static for 30 min. The cell density increased to 1.76×10^6 cells/mL two days later and most of the beads came near to confluence. The culture process was turned back to continuous agitation in the following days (Figure 1).

It was found by microscopic observation that bead-to-bead cell transfer usually occurred where confluent beads contacted with bare ones by a bridging effect (Figure 2A). Even so, that bridging force was not strong enough to withstand the shear effect generated by mechanical agitation. This guaranteed that no large bead-bead aggregate formed during the culture process. In the other hand, the beads detached from the bridge might contact confluent beads again (Figure 2b). This made a more even bead-to-bead cell transfer possible.

However, it can be ruled out that bead-to-bead transfer was due to the attachment of free cells, either as growth of suspended cells or cells sloughing off from the confluent beads by agitation, because cells in mitosis would turn to a round shape and could be easily detached from the microcarriers by slow agita-

Figure 2. Bead-to-bead transfer of Vero cells grown on recycled microcarriers. A: Cell transfer occurred where bare beads contacted with confluent beads by a bridging effect (100 ×); B: Bridged beads detached by agitation and became free again.

tion. The detached cells usually re-attached onto the microcarriers, particularly onto bare ones.

Bead-to-bead cell transfer using unused Cytodex-3 microcarriers

Vero cells were cultured on 0.4 g Cytodex-3 microcarriers in 200 mL culture medium with initial density of 1.2×10^5 cells/mL. 4 days later, the culture reached about 90% confluence. The culture continued with addition of 0.8 g new beads. However, the culture did not show significant growth the next day. Only a few fresh beads had some cells, while others were still bare. The culture process was then changed into the intermittent mode of agitation, i.e., agitated for 1 hr and then kept static for 30 min. The cell density in-

Figure 3. Cytodex-3 microcarrier cultures of Vero cells.

creased greatly from 9.1×10^5 cells/mL to 1.59×10^6 cells/mL (Figure 3) one day later. With the process development, bare beads became less, more and more fresh beads had cells transferred from the confluent ones and showed abundant growth. On the 8th day, no obvious difference could be distinguished between the later added fresh microcarriers and the original ones.

Obviously, the attachment of free cells did not contribute much to bead-to-bead cell transfer in the culture process. It was the contact of bare beads with confluent ones that led to the cell migration from one bead to another.

Conclusion

The subculturing method is one of the critical factors to be considered in the scale-up of microcarrier cultures of anchorage-dependent animal cells. Bead-to-bead cell transfer provides an attractive solution to the scale-up operation. Not only does it minimize the physiological damage done by traditional cell harvesting methods, but it also makes the scale-up process easier to perform and the microcarrier technology more promising in the commercial production of biological products.

The operational mode is critical to successful bead-to-bead cell transfer. Intermittent agitation allows the beads to fully contact with each other and the cells to migrate from confluent microcarriers to bare ones. An automatic culture system allowing intermittent operation should be developed to make full bead-to-bead contact while no severe bead aggregation

will be formed in the culture. The study also suggested that recycled Cytodex-3 microcarriers can support the growth and transfer of Vero cells as well as new ones. However, the problem of dissynchronization should also be considered before this technology can finally be put into practice.

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