Justification of continuous packed-bed reactor for retroviral vector production from amphotropic $\Psi$CRIP murine producer cell

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Received 11 January 2000; accepted 13 June 2000

Key words: anchorage-dependent cell, cell culture, packed-bed reactor, retroviral vector, viral production.

Abstract

To identify a plausible large-scale production system for retroviral vector, three culture systems, i.e., batch culture with medium exchange, microcarrier culture, and packed-bed reactor culture were compared. In batch cultures with medium exchange, high cell concentrations were maintained for about a month, and the harvested retroviral titer remained constant. In microcarrier cultures, although cell growth was rapid, the retroviral titer was unexpectedly low, suggesting that the low titer was due either to serious damage to the retroviral vector or to a reduction in the production rate of retroviral vector, caused by mechanical shear forces. Although the retroviral titer (maximum titer, $1.56 \times 10^6$) in the packed-bed reactor was a little bit lower than that obtained in the batch culture with medium exchange (maximum titer, $1.91 \times 10^6$), continuous production made it possible to increase the cumulative titer up to 16-fold of that from the batch culture with medium exchange. Moreover, as the packed-bed reactor system requires less labor and shows excellent volumetric productivity in comparison to batch cultures with medium exchanges, it will be an appropriate production system for retroviral vector in large quantities.

Introduction

The theory behind gene therapy is rather simple. However, many hurdles exist to making it work in practice because of difficulty in delivering transgenes efficiently and obtaining sustained expression of these genes. Among various gene delivery systems, retroviral vectors are the most widely used vehicles for stable gene delivery and expression in the target cell (Miller, 1992), despite the limited application of the retrovirus to dividing cells (Jolly, 1994). As the number of clinical trials has increased drastically since the first trial in 1990, a large amount of retroviral vectors is needed for gene therapy to be conducted on a large scale. To meet the burgeoning demand, the large-scale culture of producer cell is necessary. Although many studies have focused on the construction of high-producer cell lines and tissue-specific viral vectors (Ory et al., 1996; Parente and Wolfe, 1996), studies on downstream processing, such as the production process, are scarce (Andreadis et al., 1999). Only limited information is available on the factors affecting retroviral production (Lee et al., 1996; Lee et al., 1998; Shen et al., 1996). Short half-lives of retroviral vectors at 37 °C, i.e., 2–8 hr (Lee et al., 1998; Shen et al., 1996; Le Doux et al., 1999), is one of the problems to consider for large-scale production. In addition, since the producer cells are anchorage-dependent, a high surface-to-volume ratio of the culture vessel is desirable for batch cultures as well as for continuous or semi-continuous cultures. For this reason, we are interested in comparing microcarrier culture systems, packed-bed reactor systems, and hollow fiber reactor systems.

The microcarrier culture system has been widely used because of a large surface area-to-volume ratio and the homogeneous environment (Varani et al.,
1983; Wiedell et al., 1984), although it exhibits shear stress as well as slow initial cell attachment rates at high agitation speeds (Croughan et al., 1987; Hu et al., 1985). On the other hand, the packed-bed reactor system has several advantages, such as yielding high cell densities and productivities of target protein, protection from mechanical shear, easy medium exchange and product separation, continuous removal of inhibitory metabolites and easy scale-up potential. Moreover, it is not impossible to overcome the limitations of the packed-bed reactor system, such as poor oxygen transfer, excessive biomass build-up, and difficulties in recovering biomass from the bed (Griffiths and Looby, 1991). Therefore, many researchers are attracted by this system for anchorage-dependent cells (Chiou et al., 1991; Looby and Griffiths, 1988, Park and Stephanopoulos, 1993). The Hollow fiber reactor system also allows us to obtain very high cell densities (Oh et al., 1994). One attractive feature of the hollow fiber reactor system is the easy set-up of simultaneous separation of the target product, especially when a serum-free medium is used. However, hollow fiber reactor systems are more frequently used for suspension cells such as hybridoma cells than for anchorage-dependent cells, largely because fiber walls are not conductive to cell attachment (Hu and Peshwa, 1991; Strand and Quarles, 1984). For this reason, the hollow fiber reactor system is excluded in this comparison.

In this paper we compared the three culture systems to determine which system is the most conducive to large-scale production of retroviral vectors. We show that the packed-bed reactor system is the most promising approach for continuous retroviral vector production.

Materials and methods

Cell lines and culture conditions

The retroviral vector MFG-lacZ and ψCRIP/MFG-lacZ producer cell have been described elsewhere (Dranoff et al., 1993; Ohashi et al., 1992). NIH3T3 cells were used to determine MFG-lacZ retroviral vector titers. Both ψCRIP/MFG-lacZ and NIH3T3 cells were cultured in Delbecco’s modified Eagle medium (DMEM) supplemented with 10% heat-inactivated calf serum in a humidified incubator with 5% CO2 in gas phase at 37 °C.

Batch culture with medium exchange

The producer cells (1.0 ± 0.1 × 10^5 cells) were inoculated into six-well culture plates by using 2 ml of DMEM containing 10% calf serum per well. After three days of cultivation, the culture temperature was reduced to 34 °C, and the media were exchanged over the culture period as follows: (1) daily total replacement of the medium with the fresh medium (2 ml day^{-1}); (2) daily one-half replacement of the medium with fresh medium (1 ml day^{-1}); and (3) one-half replacement of the medium with fresh medium every 12 hr (1 ml 0.5 day^{-1}). After trypsinization of the cells, the viable cell concentrations were determined using a hemocytometer by the trypan dye exclusion method (Kaltenbach et al., 1958).

Microcarrier culture

The producer cells were cultured on cytodex-3 beads in a spinner flask (Bellco, Vineland, NJ) in 100 ml of culture volume under 5% CO2 humidified atmosphere at 37 °C. The concentration of the beads was 0.3 g 100 ml^{-1}, corresponding to 810 cm^2 of surface area. The agitation speed of the impeller in the spinner flask was 25~30 rpm. To prevent the microcarriers from sticking to the vessel walls, the spinner flask was siliconized prior to use. Microcarriers were hydrated and washed with Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) and autoclaved before use. Sterile microcarriers were washed with the culture medium before they were transferred to the culture vessels. The producer cells (1.0 ± 0.1 × 10^7 cells) were inoculated into the culture medium containing cytodex-3 with 2 min, intermittent agitation (40 rpm) every 30 min. during the initial 6 hr. The agitation speed was then kept at 25~30 rpm. As for the batch culture with medium exchange, the medium was replaced with fresh medium after two days of cultivation. The medium exchange rate was one-half of the culture volume per day. The concentrations of attached cells were determined by the nuclei count method using a hemocytometer, after incubation of the samples for 1 hr at 37 °C with 0.1% (v/v) crystal violet solution in PBS containing 0.1M citric acid. The viable cell concentrations were determined taking into account the viable cells measured by trypan dye exclusion method after trypsinization of the cells.

Packed-bed reactor culture

A mini packed-bed reactor was designed for this work (Figure 1). All the vessels were connected with gas permeable silicon tubing (MR-96400, Cole-Parmer