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LARGE-SCALE PRODUCTION OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN CELL CULTURE ON MICROCARRIERS

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Summary. – Large amounts of infectious bovine rhinotracheitis (IBR) virus was produced in Madin-Darby bovine kidney (MDBK) cells in suspension culture on the microcarriers Gelaspher M. Virus yields ($TCID_{50}$ per ml) were approximately 10-times higher as compared to clasical stationary cell culture.

Key words: infectious bovine rhinotracheitis virus; MDBK cells; microcarriers; large-scale production

Introduction

The principle of cultivation of cells on microcarriers is simple: the cells adhere, grow and multiply on the surface of beads. The best results are obtained using suspensions of microcarriers floating in gently mixed culture medium. In this way the microcarrier beads do not sediment, they keep continuously moving due to the mixing of medium. This arrangement has many advantages. It fulfils the demands for a solid support which is necessary for the growth and division of majority of normal tissue cells. The monitoring of cultivation conditions and the cell sampling are easy. The utilization of nutrients from culture media is 4–6 times more efficient with microcarriers than without them, what results in marked saving of expensive culture media. The risk of bacterial contamination is reduced. Also the man-power is saved; there are even reports on full automatization of cell cultivation on microcarriers (van Wezel, 1967; Reuveny, 1985).

The advantages of using gelatin microcarriers for the cultivation of fibroblasts and endothelial cells were described by Wissemann and Jacobson (1985). The growth of MDBK and other types of cells on three different commercial gelatin microcarriers was compared by Varani *et al.* (1986). MDBK cells grew on all types of microcarriers tested with a high percentage of viable cells, the Ventregel beads having the best properties.

The use of large-scale cell cultivation on microcarriers for the preparation of viral vaccines was described by Arathoon and Birch (1986). In our work we used

a microcarrier Gelaspher M for suspension cultivation of MDBK cells and finally for a large-scale production of IBR virus. This virus product might serve as a suitable starting material for preparation of formalized IBR vaccine.

Materials and Methods

Viruses. The IBR-R-6 and the IBR-BK-5-PTOB virulent strains of IBR virus, causing respiratory disease in cattle after intranasal application were used.

Cells. MDBK cells were cultivated in Eagle's minimal essential medium (MEM, ÚSOL, Prague) supplemented with 10 % inactivated calf serum.

Microcarriers Gelaspher M were developed and manufactured by Lachema (Brno) on the basis of chemically modified gelatin. For cultivation of MDBK cells a concentration of 30 ml of Gelaspher M per one liter of cultivation medium was used. The optimal size of microcarrier particles for MDBK cells (data not shown) was in the range of 150–200 μm. One ml of the microcarrier suspension corresponded to approximately 260 000 particles with surface area 250 cm^2.

Bioreactors. For suspension cultivation on microcarriers two types of bioreactors were used: (a) special 5 liter volume bottle from neutral glass with stirrer driving unit on the bottle neck, the whole fixed on a stand at an angle of 75 ° (Bioveta, Nitra); (b) 20 liter Laboratory bioreactor SLF 20 (DAK Slušovice) adapted for cell cultivation.

Cultivation on microcarriers. The cell inoculum for large-scale cultivation on microcarriers was prepared in stationary cultures in 1 200 ml Roux flasks. Cell monolayers were trypsinized, washed twice with PBS, pelleted by centrifugation and resuspended in fresh medium. The concentration of

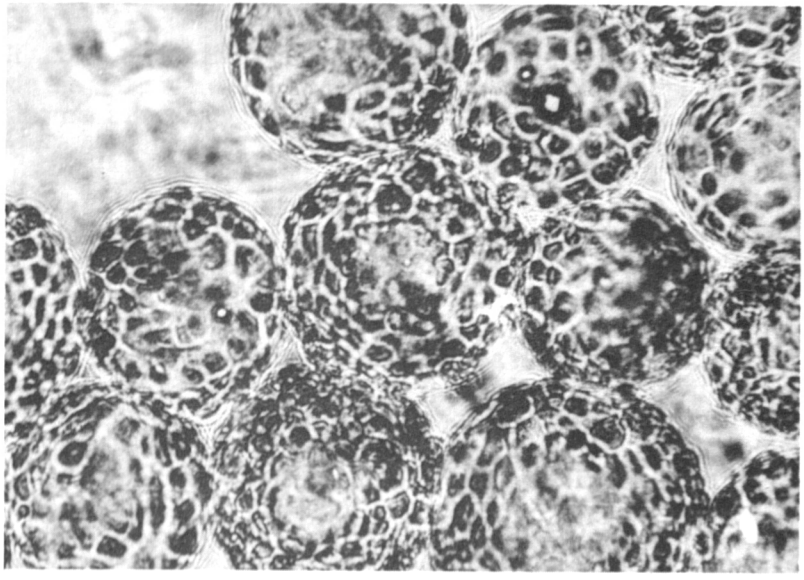

Fig. 1
Growth of MDBK cells on microcarriers after 48 hr cultivation

viable cells was estimated after trypan blue staining. Since there is no difference in cultivation of cells in two types of bioreactors described above (except the volume of cultivation medium), we describe in detail the technology of cultivation only in the bioreactor SLF 20 using 10 liter volume of cultivation medium.

First, the suspension of cells and microcarriers in cultivation medium was prepared. A 300 ml volume of suspension of Gelaspher M in PBS was mixed with 1.5×10^9 MDBK cells and added to 3 liters of Eagle's MEM with 10 % ICS. After shaking the suspension was transferred into the bioreactor and heated to 37 °C. Then the suspension was stirred for 8–12 hr discontinuously: 1 min stirring a 50 rpm and 30 min without stirring. After this period about 80–90 % of cells attached to the bead surface. This attachment period was controlled in samples of microcarrier suspension along with pH and microbiological control. Then 7 liters of cultivation medium (37 °C) was added to the bioreactor and the suspension was kept continuously stirred at 50 rpm. The cell growth on beads was controlled in samples after 24, 48 and 72 hr of cultivation. As a rule, about 90 % of beads were covered by cell monolayers after 2 days of cultivation.

Virus infection of cell microcarrier culture. The stirring was stopped, microcarriers settled down and the medium was exhausted. Then microcarriers were washed with 5 liters of PBS and the virus infection was carried out as follows. IBR virus was diluted in 2 liters of cultivation medium without serum (37 °C) to reach the multiplicity of infection of 0.1 PFU per cell and added to the washed cells. This suspension was stirred at 50 rpm for 1 hr at 37 °C. Thereafter 8 liters of complete cultivation medium was added and the incubation continued. Samples of microcarrier suspension were taken at various times p.i. and the cells on beads were microscopically examined for the presence of CPE. Also samples of cultivation medium were taken for monitoring the infectious virus titer. When CPE was complete in all cells and the virus had to be harvested, the stirring was stopped, microcarriers were left to settle down and the cultivation medium was exhausted and saved.

Virus titration. Infectious titer ($TCID_{50}$/ml) of IBR virus was estimated in tube cultures of MDBK cells infected with serial tenfold dilutions of virus material. The CPE was read 5–7 days p.i.

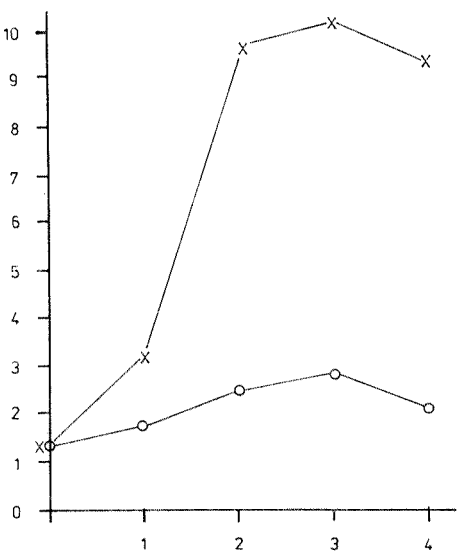

Fig. 2
Growth curves of MDBK cells on microcarriers and in stationary cultivation
x – microcarriers; o – stationary culture. Abscissa: time (days); ordinate: cell concentration (cell number/ml$\times10^5$).

Results and Discussion

Up to 80–90 % of cells attached to the surface of the microcarrier beads during the 8–12 hr attachment period. Thereafter they started to spread out and proliferate. This phase of cell growth lasted 2–3 days under continuous stirring. The morphology of cell layers on beads was controlled daily. The gelatin microcarrier beads are transparent in the cultivation medium, so that the morphology of the attached cells could be monitored straight via a light microscope.

The growth of MDBK cells on microcarriers in the bioreactor after 2 days of cultivation are presented in Fig. 1. To this time about 90 % of beads displayed a confluent cell monolayer on their surface. The growth of MDBK cells in the bioreactor and in the classical stationary culture is compared in Fig. 2, which demonstrates the respective growth curves. The cell yields (number of cell per ml) were about 4-times higher in the bioreactor than in the stationary culture (1 200 ml Roux flasks).

Three days-old culture from the bioreactor was infected with IBR virus and the cell morphology and virus titer were monitored. The CPE of virus was visible after 2 days (Fig. 3) and became complete after 3 days (Fig. 4) p.i. The infectious virus titers in the cultivation medium from the bioreactor and the stationary culture were compared (Table 1) and the formed were found by 0.5–1.0 log higher than the latter. No difference between two virus strains tested was found.

The present results demonstrate that the Gelaspher M microcarriers are suitable for a large-scale cultivation of MDBK cells in suspension in the bioreactor and for a production of high titer infectious IBR virus. The yields of both cells and virus per ml were up to 4-times and 10-times higher, respectively in the bioreactor than in the stationary culture. The bioreactor system with the Gelaspher M microcarriers displays several additional advantages concerning time, labour and costs over the classical stationary system. The IBR virus produced in the bioreactor might serve as a suitable starting material for the production of a formalized IBR vaccine.

Table 1. Infectious titer of IBR virus propagated in MDBK cells in microcarrier and stationary cultures

No. of experiment	log $TCID_{50}$/ml	
	Microcarrier culture	Stationary culture
1	8.33	7.33
2	8.33	7.50
3	8.50	7.60
4	8.33	7.33
5	8.33	7.50
6	8.33	7.30

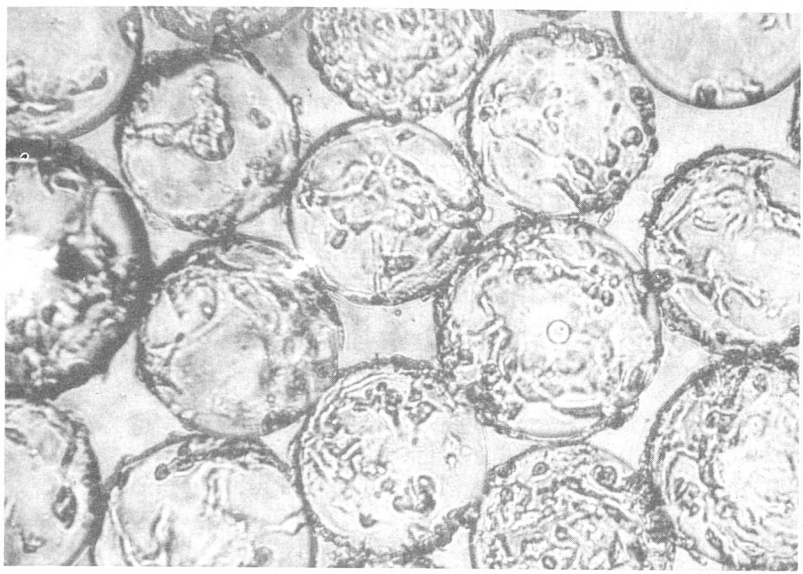

Fig. 3
CPE of IBR virus on MDBK cells 48 hr after infection

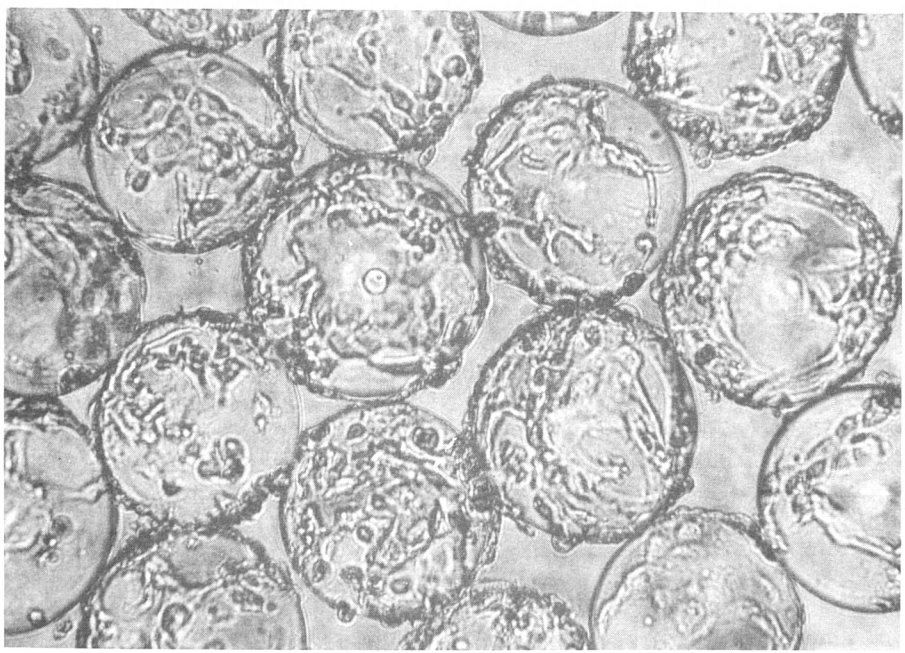

Fig. 4
CPE of IBR virus on MBDK cells 72 hr after infection

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