Cytotechnology **30:** 143–147, 1999.

High density and scale-up cultivation of recombinant CHO cell line and hybridomas with porous microcarrier Cytopore

Chengzu Xiao, Zicai Huang, Wengqing Li, Xianwen Hu, Wenlu Qu, Lihua Gao & Gaoyan Liu
Department of Cell Engineering, Institute of Biotechnology, 20 Dongdajie, Fengtai, Beijing 100071, P.R. China

Received 16 February 1998; accepted 28 November 1998

Key words: monoclonal antibody, porous microcarrier, prourokinase, recombinant CHO cells

Abstract

Using porous microcarrier Cytopore and a low-serum medium supplement BIGBEF-3, we have successfully cultivated recombinant CHO cell line CL-11G producing prourokinase and hybridomas producing anti-prourokinase monoclonal antibody in Celligen 1.5 or 5 L bioreactor. The cell density obtained ranged from 1 to 2×10^7 cells mL^{-1}. The yields of prourokinase and monoclonal antibody increased with increasing cell density. As the cells could spontaneously release from and reattach to porous microcarriers, it was very easy to scale-up the cultivation. Thus the bead to bead cell transfer method has been used to scale up the cultivation of CL-11G cells to a 20 L reactor-scale for the pilot production of prourokinase, and also to scale-up the culture of hybridomas for the production of monoclonal antibody for the purification of prourokinase.

Abbreviations: CHO – Chinese hamster ovary; dhfr – dihydrofolate reductase; McAB – monoclonal antibody; MTT – 3- (4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTX – methotrexate; NCS – newborn calf serum; PBS – phosphate buffer saline; pro-UK – prourokinase; rpm – revolution per minute

Introduction

The development of microcarrier culture by van Wezel in 1967 (Van Wezel, 1967) rendered the large- scale industrial culture of anchorage dependent cells possible for the production of vaccine and interferon. In 1980s, a great advance of this procedure was achieved by the development of a series of porous microcarriers (Lobby, 1990; Gotoh, 1993; Shirokaze, 1994). In a previous paper we reported that using porous microcarrier Cytopore, we successfully cultivated a genetically-engineered CHO cell line and some hybridomas to produce pro-UK and McAB (Xiao, 1996). This paper reports on our successful results in using the bead to bead cell transfer method to scale up the cultivation of both the CHO cells and hybridomas for the pilot production of pro-UK and McAB.

Materials and methods

Cell lines and media

The cell lines used were the genetically-engineered CHO cell line CL-11G producing prourokinase (Li, 1993) and the hybridoma cell lines X15 and 38-1-7 producing anti-prourokinase monoclonal antibody (McAB) (Ai, 1995). The medium for CL-11G cells was DMEM:F12 (1:1), supplemented with some amino acids, the low-serum medium supplement BIGBEF-3 (Xiao, 1996), as well as with 1% NCS, 1 μmol MTX, 10 KIU mL^{-1} aprotinin and antibiotics (50 μg mL^{-1} kanamycin, 2 μg mL^{-1} gentamycin and 25 μg mL^{-1} streptomycin). The medium for hybridomas was RPMI 1640, supplemented with 1% NCS and 0.1% peptone.

Porous microcarrier

The dry Cytopore microcarriers (Pharmacia Co., Sweden) were hydrated and swollen in PBS before culti-

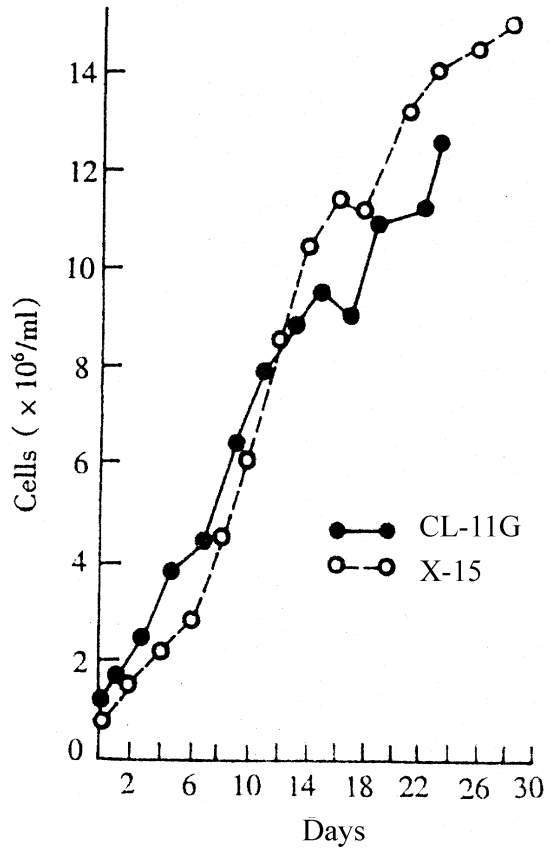

Figure 1. Proliferation curve of CL-11G cells and hybridomas X15 cultured with Cytopore carriers in a Wheaton spinner flask (200 mL).

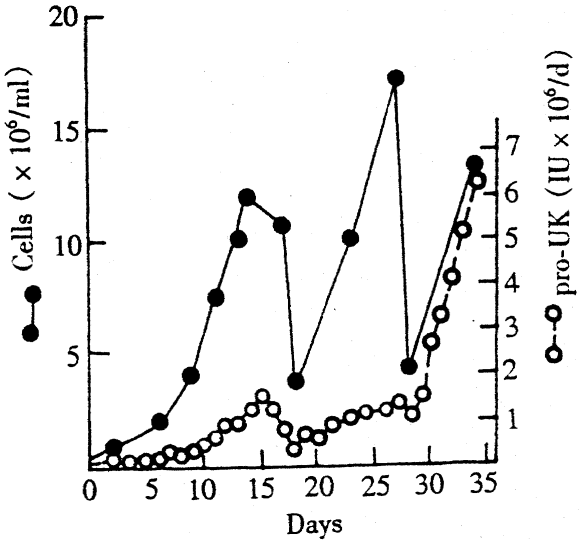

Figure 2. Proliferation curve and pro-UK production of CL-11G cells cultured with Cytopore carriers in Wheaton spinner flasks (200 mL, 700 mL).

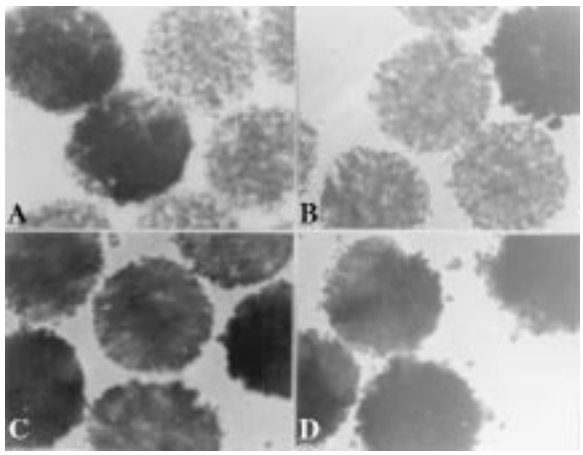

Figure 3. Scale up the culture by cells transfer from beads to beads. A) 3rd day, B) 6th day, C) 9th day, D) 12th day.

vation. After autoclaving at 121 °C for 20 min, the supernatant was removed and the microcarriers were once or twice washed with culture medium.

Culture vessels

For preliminary tests, a spinner flask (Wheaton Co., U.S.A.) was used. The stirring rate was 30–40 rpm. Then a 1.5 or 5 L Celligen bioreactor (NBS Co., U.S.A.) equipped with a modified perfusion controller (Xiao, 1994a) and a Biostat UC 20 L bioreactor (B. Braun Co., Germany) equipped with a spin filter were used.

Cell counting

The citric acid-crystal violet method was used to count cells in porous microcarriers. In order to avoid that a part of cells remained in pores, the carriers were incubated for 4–5 hr and shaken several times during incubation. At the same time, the MTT (Jia, 1993) method was also used to confirm the growth of cells inside the microcarriers.

Glucose, pro-UK and McAB assay

The methods used for measuring glucose and pro-UK were the same as those mentioned in reference (Xiao, 1994c). An ELISA assay was used for measuring the McAB concentration (Ai, 1995).

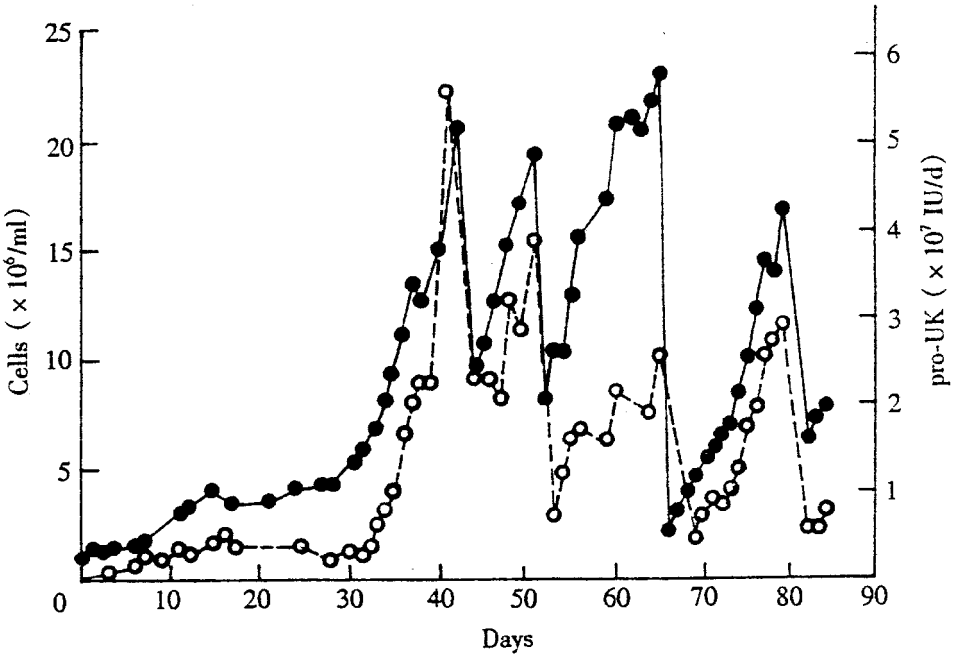

Figure 4. Proliferation curve and pro-UK production of CL-11G cells cultured with Cytopore carriers in a Celligen bioreactor (5 L).

Results

The culture of CL-11G cells and hybridomas in spinner flasks

By using a Cytopore concentration of 2.5 mg mL^{-1}, and by exchanging the medium once or twice each day, the cell density of both, the CL-11G and the hybridomas, could exceed 1×10^7 cells mL^{-1} (Figure 1). The pro-UK and McAB yields increased with increasing cell density.

Cells transfer from beads to beads

Just like for the cultures with Biosilon (Xiao, 1994c) and MC-1 (Xiao, 1994b) microcarriers, CL-11G cells could detach from Cytopore porous microcarriers spontaneously and reattach to fresh ones. So it was very easy to scale up the cultivation as reported by Kamiya (1995). When a scale up ratio of 3 was chosen, as shown in Figure 2, at day 18 we withdrew 2/3 of culture from a spinner flask (200 mL) and added the same volume of fresh medium and new microcarriers to the residual 1/3. At day 28 the whole 200 mL culture was transferred to a larger flask containing 500 mL of fresh medium and new microcarriers. At each time the final cell density could exceed 1×10^7 cells mL^{-1} (Figures 2 and 3).

Scale-up of a CL-11G cell cultivation from a spinner flask (700 mL) to the Celligen 5 L bioreactor scale

When we transferred the culture from Wheaton spinner flask to a Celligen 5 L bioreactor containing about 15 g Cytopore new microcarriers (end concentration was 4.5 mg mL^{-1}), the initial cell density was 1.06×10^6 cells mL^{-1}. After 43 days of cultivation the cells density reached 2.06×10^7 cells mL^{-1}, and the highest yield of proUK reached was 7822.6 IU mL^{-1}. At this time the volumetric productivity was 86 mg $(L\ d)^{-1}$. During the next 44 days of cultivation, we withdrew a part of the culture (about 50 to 90%) at the 44th, 52nd, 66th and 81st days, for inoculating into another bioreactor or to scale-up the culture to 20 L, by adding the same amount of vacant microcarriers. Each time except for the last time when we ended our experiment, the cell density reached was always about 2×10^7 cells mL^{-1} (Figure 4).

Scale-up cultivation of CL-11G cells from 5 to 20 L bioreactor

When we used the bead to bead cell transfer method to scale up the cultivation of CL-11G cells from the Celligen bioreactor (5 L) to the Biostat UC 20 L bioreactor, and even when we scaled up by a factor of more than 20, the cell density reached was 1.33×10^7 cells

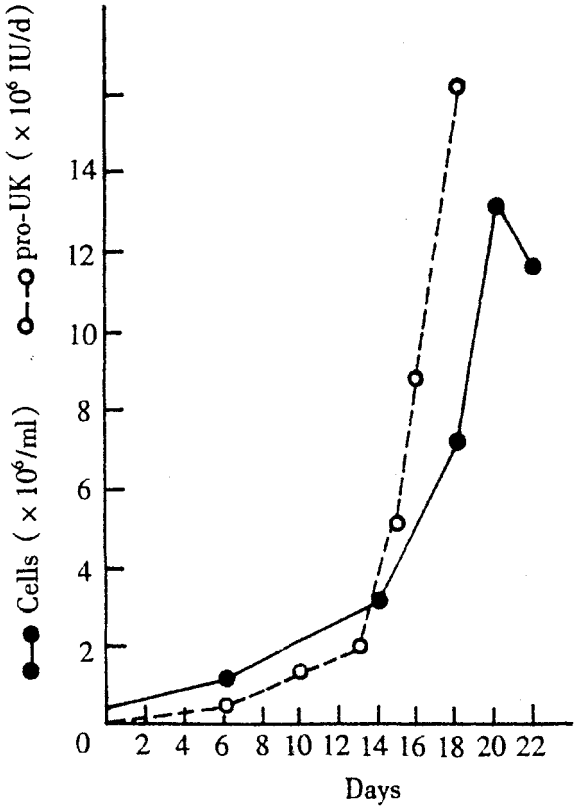

Figure 5. Proliferation curve and Pro-UK production of CL-11G cells cultivated with Cytopore carriers in a Biostat UC 20 L bioreactor.

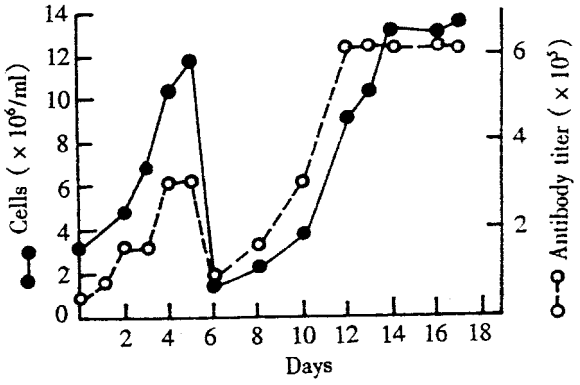

Figure 6. Proliferation curve and McAB production of 38-1-7 hybridoma cultivated with Cytopore carriers in a Wheaton spinner flask (700 mL) and transferred to a Celligen 1.5 L bioreactor.

mL^{-1}, the highest yield of pro-UK was 7358 IU mL^{-1} (Figure 5), and the highest volumetric productivity was 62.3 mg $(L\ d)^{-1}$.

Scale-up cultivation of hybridomas from Wheaton spinner flask to 1.5 L bioreactor

By using the same method we also successfully scaled up the cultivation of hybridomas from Wheaton spinner flask scale (700 mL) to the Celligen 1.5 L bioreactor scale. The cell density and the titer of McAB reached were 1.28×10^7 mL^{-1} and 6.14×10^5 mL^{-1}, respectively (Figure 6). Using this McAB for affinity chromatography, we could purify the pro-UK to a purity exceeding 95% by HPLC assay with a specific activity of 1.3×10^5 $IUmg^{-1}$ (reported elsewhere).

Discussion

Since Nilsson (1986) first developed a new type of microcarrier with a porous internal structure, a variety of porous microcarriers are commercially available (Gotoh, 1993). These porous carrier systems possess most characteristics of an ideal cell culture support (Looby, 1990). In a previous paper, we demonstrated that porous microcarriers have a lot of advantages when compared to solid microcarriers: 1) The concentration of carriers in the culture is much lower. In order to get a cell density of more than 1×10^7 mL^{-1}, about 10 g L^{-1} of sephadex microcarriers (such as MC-1, Cytodex-1), and over 50 g L^{-1} of polystyrene microcarriers (such as SH-2, Biosilon) are needed. However, only 2–3 g L^{-1} of Cytopore porous microcarriers are necessary. The reason is obvious that their porosity increases dramatically the surface area for cell attachment. The surface area of Cytopore is 2.8 m^2 g^{-1}, while that of Cytodex and Biosilon is only 6000 cm^2 g^{-1} and 225 cm^2 g^{-1}, respectively (Reuveny, 1985). 2) As the majority of cells grow inside the pores, they are protected against damage from shearing stress. Thus a much higher stirring speed can be used, i.e. 80–100 rpm were used in our experiments, and, of course, this is very beneficial for the transfer of O_2 and nutrients. 3) Cytopore porous microcarriers are not only suitable to culture anchorage-dependent cells such as CHO cells, but they are also suitable to culture anchorage-independent cells such as hybridomas. On the contrary, solid microcarriers are only suitable to culture anchorage-dependent cells. 4) Because the majority of the cells is located inside the pores, the concentration of suspended cells rather low. In addition, the cells cultured inside the porous microcarriers are less dependent on attachment factors, wherefore the serum concentration can be reduced. This is a very

important point with respect to downstream processing. This time an another advantage is found, that the bead to bead cell transfer method can also be used for scaling up the cultivation, allowing a scale up ratio of more than 20. This means that only three steps (5, 50 and 1000 L) are necessary for scaling up our culture to 1000 L. Therefore, we consider that the use of porous microcarriers for the large-scale cultivation of genetically-engineered cells for the production of recombinant products, such as prourokinase, is a very prospective approach, and will have its place in industry.

Acknowledgments

This work was supported by China National Center of Biotechnology Development. We would like to thank Pharmacia Co. for their samples and their financial support.

References

Ai X, Ye J, Xiao C and Zhang Z (1995) Preparation and characterization of monoclonal antibodies against uPA and their application for purification of uPA. Bull Acad Mil Med Sci 19: 248–253.

Gotoh T, Honda H, Shiragami N and Unno H (1993) A new type porous carrier and its application to culture of suspension cells. Cytotechnol 11: 35–40.

Jia X and Xiao C (1993) MTT method for the estimation of the number of cells cultivated in microcapsules. Bull Acad Mil Med Sci 17: 207–211.

Kamiya K, Yanagido K and Shirokaze J (1995) Subculture for large scale cell culture using macroporous microcarrier. In: Beuvery EC, Griffiths JB and Zeijlemaker WP (eds) Animal Cell Technology: Development towards the 21st Century (pp. 759–763). Kluwer Acad Publishers, The Netherlands.

Li F, Li J, Li X, Lin J and Yu W (1993) High-level expression of pro-urokinase cDNA in Chinese hamster ovary cell line (1). Bull Acad Mil Med Sci 17: 89–92.

Looby D and Griffiths B (1990) Immobilization of animal cells in porous carrier culture. TIBTECH 8: 204–209.

Nilsson K, Buzsaky F and Masboch K (1986) Growth of anchorage-dependent cells on macroporous microcarriers. Bio/Technol 4: 989–990.

Reuveny S (1985) Microcarriers in cell culture: Structure and applications. Adv Cell Culture 4: 213–247.

Shirokaze J, Nogawa M and Ogura R (1994) High density culture using macroporous microcarrier. In: Spier RE and Griffith B (eds) Products for today, prospects for tomorrow (pp. 261–263). Oxford, Butterwerth-Heinemann.

Van Wezel AL (1967) Growth of cell-strains and primary cells on microcarriers in homogeneous culture. Nature 216: 64–65.

Xiao C, Zhou H and Huang Z (1994a) An autocontroller system for cell perfusion cultivation on microcarrier. Chinese Patent, ZL 91208893.1, 1994-09-14.

Xiao C, Huang Z, Liu F, Guo Z and Gao L (1994b) High density cultivation of genetically-engineered CHO cell lines with microcarrier culture systems. Chin Med Sci J 9: 71–74.

Xiao C, Huang Z, Zhang Z, Ye J, Gao L, Guo Z, Cheng D, Zhou H and Kong W (1994c) High density cultivation of a recombinant CD-1 cell line producing prourokinase using a Biosilon microcarrier culture system. Chin Med Sci J 9: 203–208.

Xiao C, Huang Z, Li W, Qu W, Gao L and Liu G (1996) High density cultivation of recombinant CHO cell line and two strains of hybridomas with porous microcarrier Cytopore. Bull Acad Mil Med Sci 20: 191–194.

Address for correspondence: Chengzu Xiao, Department of Cell Engineering, Institute of Biotechnology, 20 Dongdajie, Fengtai, Beijing 100071, P.R. China