

Dialysis-based Bioreactor System for the Production of Chimeric Antibodies: an Alternative to Ascites Production in Mice

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Abstract: We tried to produce two chimeric antibodies to tumor-associated antigens using a new commercially available bioreactor system, miniPERM. The culture apparatus is composed of two modules: a 40 ml disposable cell culture chamber for antibody production and a 550 ml medium reservoir for nutrient supply. The two modules are separated from each other by a dialysis membrane which allows for the passage of low molecular mass nutrients and metabolites. Depending on the individual properties of the transfectoma cells cultured, cell densities of more than 10×10^6 cells per ml and chimeric antibody concentrations of more than 250 μ g per ml can be obtained in the new miniPERM bioreactor. On average, 16 mg (range: 8.3–24.4 mg) could be produced within 2 weeks. The culture method is a useful alternative to the *in vivo* production method in mice.

Key words: Chimeric antibody production, In vitro culture method, MiniPERM

Recently, Vivascience have marketed a bioreactor system, called miniPERM, that relies on a dialysis membrane which allows for the separation of cell growth and base medium compartments.⁷ This unit is primarily designed to cater for the production of mAb/ChAb in the 10–100 mg range within two weeks. It is relatively inexpensive and simple to operate.⁸ In this paper, we tried to produce two different ChAbs to carcinoembryonic antigen (CEA) and MK-1 antigen using the miniPERM bioreactor.

Materials and Methods

Medium. All transfectoma cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma Chem Co., St. Louis, MO, USA) with 4.5 g glucose and 3.7 g sodium bicarbonate per liter supplemented with 10% fetal calf serum (FCS), 100 U/ml of penicillin and 100 μ g/ml of streptomycin.

Transfectoma cell lines. The following transfectoma cell lines, recently generated by ourselves, were tested in this study: Ch F11-39 producing a chimeric antibody (IgG1) specific for CEA^{9,10}; and Ch FU-MK-1 producing a chimeric antibody (IgG1) specific for MK-1.^{5,11}

Tumor cell lines. Two human gastric cancer cell lines, MKN-45 (CEA- and MK-1-expressing) and MKN-74 (CEA-non-expressing and MK-1-expressing), were maintained in DMEM containing 10% FCS and the antibiotics.

MiniPERM™ culture conditions. The miniPERM™ bioreactor (Heraeus Instruments GmbH, Osterode, Germany) was used in accordance with the manufacturer's instructions. The production module and the nutrient module were pretreated with the relevant base medium for 24 h prior to a run. The transfectoma cells (2×10^5 each) for seeding were resuspended in 35 ml with DMEM supplemented with 10% FCS. The cells were then drawn into a 50-ml syringe and then were gently injected into a lower connector port whilst the upper port is open to allow for the displacement of air. All production module ports were then closed and the nutrient drum filled with a minimum of

350 ml of medium. A small sample (200 μ l) of cells was taken from the production module regularly to assess the concentration, viability and health of the cells. Generally, the nutrient module medium was changed every 1–2 days. After a 14-day culture, less than 35 ml of culture media including cells could be harvested from the production module to assess the total amount of the ChAbs produced.

Determination of the ChAb concentration. Quantitation of ChAbs was carried out by means of a solid-phase enzyme immunoassay using 96-well polyvinyl chloride plates coated with an affinity purified goat anti-human IgG antibody and a biotinylated goat anti-human γ -chain antibody as the tracer.⁹ A purified human IgG1(κ) myeloma protein was used as the standard.

Antibody binding assay. To determine the antibody binding to the cells, flow cytometry was also performed as described previously.⁵ Briefly, aliquots of 1×10^6 tumor cells, washed with PBS, were incubated with each ChAb or Control human IgG at 1.5 μ g/ml in Dulbecco's PBS containing 5% normal goat serum on ice for 1 h. The cells were washed with Dulbecco's PBS containing 5% normal goat serum and were incubated with fluorescein-conjugated goat F(ab')₂ anti-human IgG at 10 μ g/ml on ice for 30 min. After washing with PBS, the stained cells were analyzed using a FACSCalibur (Becton Dickinson, San Jose, CA). The percentages of positive cells and the mean fluorescence intensity were determined using the Cell Quest computer software.¹¹

Results

Growth kinetics and ChAb production. Depending on the individual properties of the two transfectomas used, the results obtained differed considerably. The typical growth kinetics and antibody production rate of the Ch F11-39 cells are shown in Fig. 1. Fast cell growth resulted in a density of 13.5×10^6 cells/ml with a viability of 62% and an antibody concentration of 270 μ g/ml on day 8. In another experiment in which the Ch FU-MK-1 cells were cultured for 14 days, 88 μ g/ml of ChAb were produced by 11.3×10^6

cells with a viability of 55% on day 9 (data not shown). The summary of the results obtained with two transfectoma cells is presented in Table 1. The antibody yields of Ch F11-39 and Ch FU-MK-1, obtained in the final harvest at the end of the culture period, were 12.2 and 4.2 mg, respectively (Table 1). These results clearly show that the new miniPERM bioreactor system allows for the production of ChAbs in high concentrations by transfectoma cells.

Binding of ChAbs to tumor cells. In order to test whether the ChAbs produced by this

new method were fully active, we analyzed their reactivities with two human tumor cell lines by flow cytometry. As summarized in Table 2, Ch FU-MK-1 significantly bound to both MK-1-expressing gastric tumor cell lines, while Ch F11-39 reacted only with the CEA-expressing MKN-45 cells but did not with the CEA-non-expressing MKN-74 cells. These results, including the mean fluorescence intensity (Table 2), are entirely consistent with our previous data for the ChAbs obtained from mouse ascitic fluid.¹¹⁾

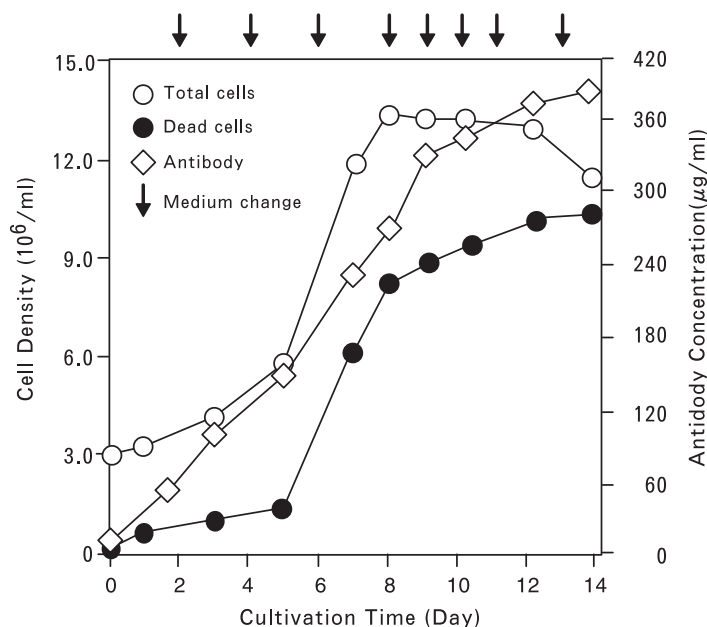


Fig. 1. Cell density and antibody production during the miniPERM culture of cells from the transfectoma cell line Ch F11-39.

Table 1. Cell densities, antibody concentrations and antibody yields obtained with two transfectoma cell lines during a 14-day culture

Transfectoma Line	Ig class	Medium type	Max. cell density ($\times 10^6$)	Max. Ab conc. $\mu\text{g}/\text{ml}$	Total harvest vol. (ml)	Total Ab harvest (mg)
Ch F11-39	IgG1 (κ)	DMEM/10% FCS	13.5	381	32	12.2
Ch FU-MK-1	IgG1 (κ)	DMEM/10% FCS	11.3	126	33	4.2

Table 2. Flow cytometric analysis of reactivity of two chimeric antibodies with two human cancer cell lines

Antibody	% Positive cells ^a	
	MKN-45 (CEA ⁺ , MK-1 ⁺)	MKN-74 (CEA ⁻ , MK-1 ⁺)
Ch F11-39	98.8 (733.6)	1.8 (10.4)
Ch FU-MK-1	95.8 (742.9) ^b	88.4 (163.2)
Control human IgG	0.3 (11.3)	0.8 (22.3)

^a Cells were incubated with Ch F11-39, Ch FU-MK-1, or control human IgG1. After washing, the bound antibody was detected with fluorescein-conjugated goat F(ab')₂ anti-human IgG.

^b Numbers in parentheses represent the mean fluorescence intensity (arbitrary units).

Discussion

This paper describes a new and easy to handle reusable bioreactor, miniPERM, for the high-density culture of hybridoma/transfectoma cells. The culture apparatus is composed of two modules: a 40 ml disposable antibody-production module and a 550 ml medium-supply module, which are separated from each other by a dialysis membrane which allows for the passage of low molecular mass nutrients and metabolites.⁷⁾ The monoclonal/chimeric antibodies are produced and enriched in the production module. The outer part of this module is made from a thin gas-permeable silicone rubber membrane allowing exchange of gases (oxygen and carbon dioxide).⁷⁾

Due to its simplicity, the handling of the miniPERM is much easier and the costs of antibody production are considerably lower than other established *in vitro* antibody production techniques (stationary culture, roller bottle culture, spinner culture, hollow fiber culture, etc.).¹²⁾⁻¹⁵⁾

The results presented herein show that it is possible to culture transfectoma cells in high density and thereby produce ChAbs in concentrations comparable in quantity and quality to those produced in the form of ascitic fluid in live mice.⁹⁾ On average, 8.2 mg (range: 4.2–12.2 mg) of ChAbs in a volume of 32–33 ml can be produced within 2 weeks.

Although the absolute antibody concentration is not as high as in ascitic fluid, the concentration of ChAbs per mg of total protein (i.e., the 'specific antibody content') is in the same range as that observed in ascitic fluid (data not shown). In this respect, the miniPERM culture supernatant can be considered almost equivalent to ascitic fluid.

When the binding reactivity with human gastric tumor cell lines was tested by flow cytometry, the ChAbs obtained showed the same specificity and affinity at equal antibody concentrations as those from the ChAbs derived from ascitic fluid.¹¹⁾ As a result, in terms of their properties the ChAbs produced in the new modular miniPERM were indistinguishable from the ChAbs prepared from ascitic fluid or from the supernatant of conventional stationary culture. Based on the above findings, this new *in vitro* culture method is therefore considered to be a useful alternative to the *in vivo* production method in mice.

References

- 1) Köhler, G. and Milstein, C.: Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, 256 : 495-497, 1975.
- 2) Kuroki, Ma., Ueno, A., Matsumoto, H., Abe, H., Li, T., Imakiire, T., Yamauchi, Y., Uno, K., Shirota, K., Shibaguchi, H. and Kuroki, M.: Significance of tumor-associated antigens in diagnosis and therapy of cancer: an overview. *Anticancer Res.*, 22 : 4255-4264, 2002.
- 3) Kuroki, Ma., Shibaguchi, H., Imakiire, T., Uno, K., Shirota, K., Higuchi, T., Shitama, T., Yamada, H., Hirose, Y., Nagata, A. and Kuroki, Mo.: Immunotherapy and gene therapy of cancer using antibodies or their genes against tumor-associated antigens. *Anticancer Res.*, 23 : in press, 2003.
- 4) Morrison, S.L.: Transfectomas provide novel chimeric antibodies. *Science*, 229 : 1202-1207, 1985.
- 5) Arakawa, F., Yamamoto, T., Kanda, H., Watanabe, T. and Kuroki, Ma.: cDNA sequence analysis of monoclonal antibody FU-MK-1 specific for a transmembrane carcinoma-associated antigen, and construction a mouse/human chimeric antibody. *Hybridoma*, 18 : 131-138, 1999.
- 6) Marx, U.: Membrane-based cell culture

technologies: a scientifically and economically satisfactory alternative to malignant ascites production for monoclonal antibodies. *Res. Immunol.*, 149 : 557-559, 1998.

- 7) Falkenberg, F.W., Weichert, H., Krane, M., Bartels, I., Palme, M., Nagels, H.O. and Fiebig, H.: In vitro production of monoclonal antibodies in high concentration in a new and easy to handle modular minifermenter. *J. Immunol. Methods*, 179 : 13-29, 1995.
- 8) Bruce, M.P., Boyd, V., Duch, C. and White, J.R.: Dialysis-based bioreactor systems for the production of monoclonal antibodies - alternatives to ascites production in mice. *J. Immunol. Methods*, 264 : 59-68, 2002.
- 9) Arakawa, F., Haruno, M., Kuroki, Ma., Kanda, H., Watanabe, T., Misumi, Y. and Matsuoka, Y.: Construction and expression of two mouse-human chimeric antibodies with high specificity and affinity for carcinoembryonic antigen. *Hybridoma*, 12 : 365-379, 1993.
- 10) Senba, T., Kuroki, Ma., Arakawa, F., Yamamoto, T., Kuwahara, M., Haruno, M., Ikeda, S. and Matsuoka, Y.: Tumor growth suppression by a mouse/human chimeric anti-CEA antibody and lymphokine-activated killer cells in vitro and in SCID mouse xenograft model. *Anticancer Res.*, 18 : 17-24, 1998.
- 11) Yamamoto, T., Arakawa, F., Nakamura, K., Senba, T., Tomita, Y., Ikeda, S. and Kuroki, Ma.: Enhanced antitumor activity of a combination treatment with a mouse/human chimeric anti-MK-1 antibody and lymphokine-activated killer cells in vitro and in a severe combined immunodeficient mouse xenograft model. *Cancer Immunol. Immunother.*, 48 : 165-171, 1999.
- 12) Falkenberg, F.W., Hengelage, T., Krane, M., Bartels, I., Albrecht, A., Holtmeier, N. and Wuthrich, M.: A simple and inexpensive high density dialysis tubing cell culture system for the in vitro production of monoclonal antibodies in high concentration. *J. Immunol. Methods*, 165 : 193-206, 1993.
- 13) Vandamme, A.M., Bulens, F., Bernar, H., Nelles, L., Lijnen, R.H., Collen, D.: Construction and characterization of a recombinant murine monoclonal antibody directed against human fibrin fragment-D dimer. *Eur. J. Biochem.*, 192 : 767-775, 1990.
- 14) Voigt, A. and Zintl, F.: Hybridoma cell growth and anti-neuroblastoma monoclonal antibody production in spinner flasks using a protein-free medium with microcarriers. *J. Biotechnol.*, 68 : 213-26, 1999.
- 15) Yazaki, P.J., Shively, L., Clark, C., Cheung, C.W., Le, W., Szpikowska, B., Shively, J.E., Raubitschek, A.A. and Wu, A.M.: Mammalian expression and hollow fiber bioreactor production of recombinant anti-CEA diabody and minibody for clinical applications. *J. Immunol. Methods*, 253 : 195-208, 2001.

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