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# Evaluation of Hollow Fiber And Miniperm Bioreactors as An Alternative to Murine Ascites for Small Scale Monoclonal Antibody Production

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# دراسة لتقييم استخدام المفاعلات البيولوجية كبديل لانتاج الاجسام المضادة احادية المنشأ الخلوي من خراج فئران التجارب

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## Abstract

The objective of this study was to compare monoclonal antibody production in hollow fiber, miniPERM bioreactor systems and murine ascites to determine the feasibility of the bioreactor system as a potential alternative to the use of mice. One hybridoma cell line was grown in hollow fiber, miniPERM bioreactor systems and in groups of 5 mice. Mice were primed with 0.5 ml pristane intraperitoneally 14 days prior to inoculation of $1X10^7$ hybridoma cells. Each mouse was tapped a maximum of three times for collection of ascites. Bioreactors were harvested three times weekly for 30 days and were monitored by cell counts, cell viability and media consumption. Time and materials logs were maintained.

The total quantity of monoclonal antibody produced in 5 mice versus the total production for the two different bioreactors (hollow fiber and miniPERM) in 30 days was as follows: cell line 2AC10E6C7 produce 158 mg vs.97.5 mg; vs 21.54 mg respectively.

Mean monoclonal antibody concentration ranged from 4.07 to 8.37 mg/ml in murine ascites, from 0.71 to 3.8 mg/ml in hollow fiber bioreactor system, and from 0.035 to 1.06 in miniPERM. Although time and material costs were generally greater for the bioreactors, these results suggest that hollow fiber and miniPERM bioreactor systems merit further investigations as potentially viable in vitro alternatives to the use of mice for small scale (< 1 g) monoclonal antibody production.

## خلاصة

هدف هذه الدراسة كانتْ أنْ تُقارنَ إنتاجَ اجسام مضادة أحادية السلالة ( monoclonal antibodies) في مفاعلات بيولوجية (أنظمة الالياف المجوّفة و miniPERM) مع انتاجها بواسطة حقن فئران بالخلايا المزاوجة المسببة لخراج يحتوي على الاجسام المضادة (ascites) لتَقْرير عمليةِ نظامِ المفاعلات كبديل محتمل إلى إستعمالِ الفئرانِ. واحد خَطّ خلية (hybridoma) نُمِي في نظام الليفِ المجوّفِ وفي نظام miniPERM كما نمي في مجموعة مِنْ 5 فئران. الفئران عُبّيتْ ب 0.5 مليلتر pristane تحت جدار البطن 14 يوم قبل تلقيحها بخلايا ال hybridoma. كُلّ فأر نُقِرَ ثلاث مراتِ كحَدّ أعلى لجمع الخراج. تم حصد المحتويات من المفاعلات البيولوجية بمعدل ثلاث مرات اسبوعيا لثلاثين يومٍ وروقبَ من خلال اعداد الخلايا الحية النشطة ومحتوى الوسط الغذائي. تم عمل سجلات للموادَ المستخدمة وزمن الزراعة والحصد.

الكمية الكليّة للجسم المضاد أحادي السلالة التي أنتجتْ في 5 فئرانِ مقابل الإنتاجِ الكليِّ لإثنان من المفاعلات البيولوجية المختلفة (الالياف المجوّفة وminiPERM) في 30 يومٍ كَانَ كالتّالي: 158 مليجرام للفئران مقابل 97.5 مليجرام للالياف المجوفة مقابل 21.54 مليجرام لل miniPERM.

تَراوحَ معدل انتاج الاجسام المضادة أحادية السلالة في الانظمة الثلاث مِنْ 4.07 إلى 8.37 ملج / مليلتر في murine ascites، مِنْ 0.71 إلى 3.8 ملج / مليلتر في نظام الالياف المجوّفة ومِنْ 0.035 إلى 1.06 ملج/مليلتر في miniPERM.

بالرغم من أن الوقتِ والكلفِ الماديةِ كَانا أكبر بشكل عام للمفاعلات البيولوجية الا ان استخدام هذه التقنيات لفترة طويلة من الزمن يقلل التكلفة تقليل معنوي.

تَقترحُ هذه النَتائجِ بأنّ انظمة الالياف المجوّفة وأنظمةِ MiniPerm bioreactor يَستحقّانِ تحقيقاتَ أخرى كبدائل خارج الجسم فعّالة فعلاً إلى إستعمالِ الفئرانِ للنطاق الضيقِ (g 1>) إنتاج جسم مضادِ أحادي السلالةِ.


## Introduction

Antibodies are glycoproteins of animal origin which provide humoral immunity and form a major part of the immunological defenses against infection by invading microorganism. Since their discovery they have been used as diagnostic reagent and more recently as therapeutic biologicals.

The most extensively used technique to date for the production of MAbs is *in-vivo* production in ascitic fluid. The ascites production of

hybridoma-derived monoclonal antibodies has been in routine use since the late 1970's following the Nobel Price winning Köhler and Milstein publication in 1975[1]. Some twenty years later, there is still debate over the practical, financial and moral issues of manufacturing monoclonal antibodies *in-vivo* compared with *in-vitro* methods. The extensive use of the ascites method is due to the fact that the technique is relatively simple and was initially inexpensive when compared to the *in-vitro* alternatives available at the time.

There has recently been a number of extremely informative articles published describing the numerous *in-vitro* products and systems available for the production of monoclonal antibodies. A number of these papers provide comprehensive data comparing the ascites production of monoclonal antibodies with the *in-vitro* alternatives available [2, 3].

The use of cell culture hollow-fiber bioreactors is well established for the production of monoclonal antibodies, recombinant protein products, viruses and viral antigens, and viable cell mass.[4-6] In such systems, the bulk of the culture medium is separated from the cell mass by means of hollow-fiber walls, allowing production of high-density cultures of viable cells (> $10^8$ cells/ml) in the extracapillary space (ECS).[7] This results in highly concentrated antibody and protein harvests.[8] Antibody harvests commonly reach 1 to 3 mg/ml in the harvested supernatant, but yields in excess of 17 mg/ml have been reported.[9,10] Yields such as these, together with their simple and relatively inexpensive operation, make hollow-fiber bioreactors an attractive means of producing antibodies from mammalian cells.

Antibody production using research-scale hollow-fiber bioreactors is generally not as efficient as using mice; quantities of antibody from a few to 100 milligrams are more often produced in shorter periods of time by mice.[11, 12] However, it is possible that hollow-fiber bioreactors can be made to support more productive cell growth and antibody production by optimizing the formulations of culture media, the configuration of the hollow-fiber bioreactor, and operating protocols.[8, 13]

Unlike homogeneous suspension cultures, in which the medium surrounding cells contains an even concentration of nutrients and growth factors, hollow-fiber membranes provide a heterogeneous environment by separating the cells from the circulating medium into

compartments of extra- and intracapillary spaces (ECS and ICS). Cells are grown in the ECS and are nourished by nutrients circulating in the ICS medium that readily diffuse across the hollow-fiber membrane. Metabolic waste products similarly diffuse away in the opposite direction and are diluted into the medium circulating in the ICS.

The MiniPerm culture vessel is a small size bioreactor made up of two connected modules designed for the culture of hybridomas and other cells in high densities. It comprises:

- a production module which is a disposable culture chamber with a volume of about 40 ml and,
- a nutrient module which is a reusable culture chamber with a volume of about 550 ml made of autoclavable plastic (polycarbonate) to which the production module is connected.

On the side facing the nutrient module, the production module is fitted with a dialysis membrane (MWCO 12.5 KD). When the production module is attached to the nutrient module, the dialysis membrane separates the two modules from each other. The other side of the production module, facing outwards, is made of a very thin silicone rubber membrane, which is permeable to $CO_2$ and $O_2$.

**Materials and Methods**

**a. Mice**

BALB/c mice were obtained from National Institute of Health, Nunthaburi, Thailand Breeding and Research Center.

**b. Cell Line**

2AC10E6C7 Cell line was used in this study: secretes a mouse $IgG_1$ antibody directed against human thyroid stimulating hormone (TSH). The cell line was maintained in RPMI culture medium containing 2.5 g/L glucose, 0.292 g/L glutamine, and 10% volume-to-volume (V/V) supplemented fetal bovine serum (FBS; Hyclone, Logan, UT) as a complete medium.

**Static Culture**

Static suspension cultures were performed in standard 25 $cm^2$ tissue culture flasks using complete medium. The cells were passed to a fresh medium three times a week. All flasks were incubated at 37°C in a $CO_2$ incubator with 5% $CO_2$ to maintain a pH between 7.0 and 7.4.

To provide enough cells in log-phase growth for the miniPERM, hollow-fiber bioreactor and for ascites production experiments, cells were expanded by standard static culture techniques in 175 $cm^2$ tissue

culture flasks (Nunclon; Nalge Nunc International, USA). Cells were counted on a hemocytometer using trypan blue dye exclusion.

**Assays**

1- All $IgG_1$ antibody products of interest were quantified by spectrometry after affinity purification.
2- The quantification of IgGs from miniPERM was done by immunoradiometric assay through immobilizing a goat anti-mouse IgG onto polystyrene tubes inner surface. Samples from miniPERM were then added followed by a radiolabelled rabbit anti-mouse IgG.

**miniPERM**

A five standard tissue culture flasks 175 $cm^2$ were maintained in complete medium for miniPERM at a concentration of 1.6 x $10^6$ cells/ml with 70% viability. These flasks were centrifuged at 400* G for 5 minutes in a tabletop centrifuge and resuspended in 40 ml complete medium for injection into miniPERM production module.

**Hollow-Fiber Cell Culture System**

Evaluations of antibody production in hollow-fiber bioreactors with 0.14 $m^2$ fiber surface area were performed with a RPMI medium containing10% volume-to-volume (V/V) supplemented fetal bovine serum (FBS; Hyclone, Logan, UT) as a complete medium. Thirty-day cell culture experiments were conducted for a hybridoma by injecting $2x10^8$ total cells (> 90% viability) into flushed hollow-fiber bioreactors. The bioreactor system shown schematically in Figure 1 was placed in $CO_2$ incubators in order to maintain a constant temperature and pH. Oxygenation occurred while the ICS medium stream passed through thin-walled silicone tubing system.

The media used in the ICS and ECS consisted of RPMI supplemented with 0% and 10% FBS, respectively. Spent ICS medium was gradually replaced by RPMI to maintain nutrients above certain levels, such as keeping glucose concentration greater than 2 mg/ml. ICS medium was circulated through the inside of the fibers and back to the ICS medium reservoir at a flow rate of 200 ml/min. Beginning on the fourth day of the experiment, 10 ml of medium containing antibody secreted by cells was harvested from the ECS every other day. The harvest was accomplished using two 10-ml syringes. One empty syringe was used to collect the harvested medium while a second

syringe containing 10 ml of fresh ECS medium was used to displace the harvested medium.

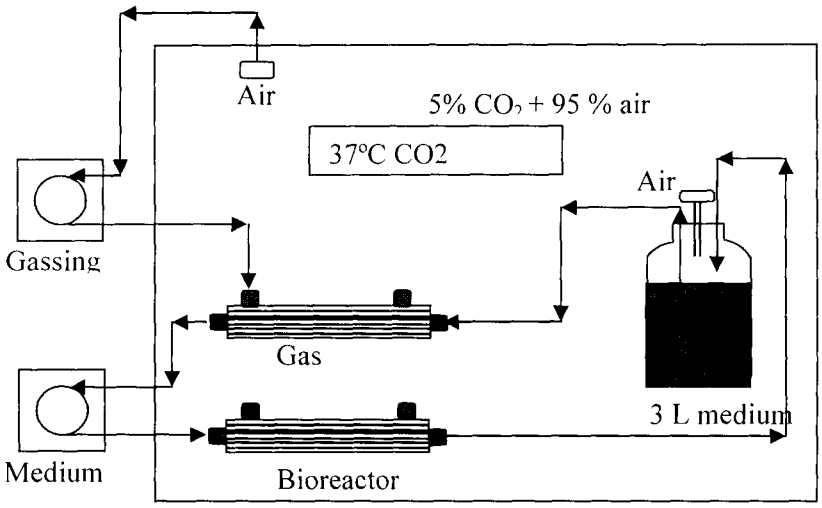


**Fig.1: Hollow fiber bioreactor system for in-vitro production of monoclonal antibodies**

**Ascites fluid**

Hybridoma cells can be grown as ascites tumors (tumor cell suspension in vivo) in the peritoneal cavity of a mouse. The prior injection of pristine (a mineral oil-like substance) appears to induce the formation of very high numbers of rapidly reproducing ascites tumors.

Mice were primed intrapretoneally with pristane two weeks before injection with hybridoma cell.

For the purification of ascite fluid, protein A Sepharose affinity chromatography was used.

**Results**

A comparative study on the production of monoclonal antibody by in-vitro technique (hollow fiber, miniPERM) and by in-vivo technique (ascetic fluid) were carried out. Table 1 shows the total yield of a monoclonal antibody (mg) and IgG range (mg/ml)of the three techniques which can be arranged ascendingly as follows: murine ascites>hollow fiber > miniPERM.

**Table I: Monoclonal antibody yield in miniPERM, hollow fiber bioreactor and murine ascites**

| | murine ascites | Hollow fiber | MiniPerm |
|---|---|---|---|
| Total yield (mg) | 158 | 97.5 | 21.54 |
| IgG range (mg/ml) | 4.07 - 8.37 | 0.71 to 3.8 | 0.035 to 0.1 |
| Mean | 5.65 | 1.953 | 0.071 |
| SD | 1.48 | 0.94 | 0.02 |
| N | 15 | 15 | 15 |

Fig. 2 shows the antibody concentration (mg/ml) obtained by the three techniques studied.

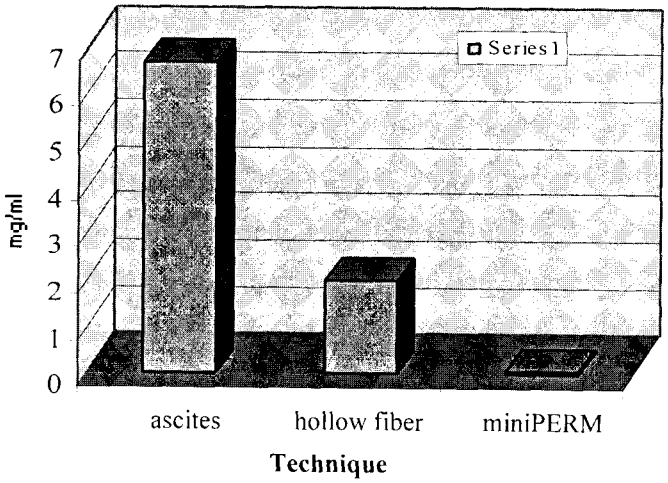


**Fig.(2): Production of monoclonal antibodies by three different techniques (mg/dl**

## Costs

In fact, the cost and productivity of the culture medium used in a hollow-fiber bioreactor and miniperm has a direct effect on the cost-efficiency of the systems for antibody production. Table II indicates estimated costs for such a system, based on the costs of culture medium and disposable hollow-fiber bioreactors (labor and facility costs are not included).

**Table II: Comparative costs of producing monoclonal antibodies using hollow fiber bioreactor and miniPERM systems**

| System type | Medium Cost ($) | Bioreactor Cost ($) | Productivity (mg MAb/L medium) | Cost Efficiency ($/mg MAb) |
|---|---|---|---|---|
| Hollow fiber | 48 | 82 | 8.13 | 1.52 |
| Miniperm | 16 | 187 | 5.37 | 1st 6 month 8.7 then 0.75 |

The cost of the medium was estimated at $4.00/L for RPMI and $0.30/ml for FBS.

## THE COST FOR MURINE ASCITES PRODUCTION

Five Balb/C mice cost 60 US$ plus approximately 60 $ for other expenses (feeding, consumables, handling, husbandry, etc...). The total cost was 120 US$.
The cost per mg MAb = 120/158 = 0.75 US$

## Conclusion

Monoclonal antibodies are widely used as diagnostic and research reagents. Their introduction into human therapy has been much slower.
In some in vivo applications, the antibody itself is sufficient. Once bound to its target, it triggers the normal effector mechanisms of the body.
In other cases, the monoclonal antibody is coupled to another molecule, for example

- a fluorescent molecule to aid in imaging the target
- a strongly-radioactive atom, such as Iodine-131 to aid in killing the target

The aim of this study was to evaluate the feasibility of producing monoclonal antibodies in bioreactors instead of murine ascites. Both bioreactors are quite sophisticated and need skills and well trained personnel to deal with.
In this study the monoclonal antibody produced seems to be within an expected range of many researchers using the same techniques [8-10]

The bioreactors are a long period running technique i. e. once settled up, the operation may last for six month at least unless the system got contamination. For that, it can be used efficiently and reliably as an alternative for ascites production. The production cost is quite comparable to the cost of that for murine ascites.

In this study, operating conditions were standardized and no attempt was made to optimize production parameters. Nevertheless, it is recognized that antibody yields could be increased by optimizing any number of operating variables, including the flow rate of the ICS medium, the frequency and volume of the ICS medium exchange, and the frequency and volume of harvest.

Increasing the flow rate of the ICS medium can raise the pressure within the system, thus enhancing nutrient exchange. In addition, because of their small molecular sizes, many useful autocrine factors could be irreversibly dialyzed away from the ECS to the ICS medium.

The handle of the Miniperm is much easier and safe than the hollow fiber which helps in prolonging the duration of culture in a system with reduced susceptibility to contamination.

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