美国健康医疗仪器国际公司

35 Weaver Street, *Scarsdale*, New York 10583. U.S.A.

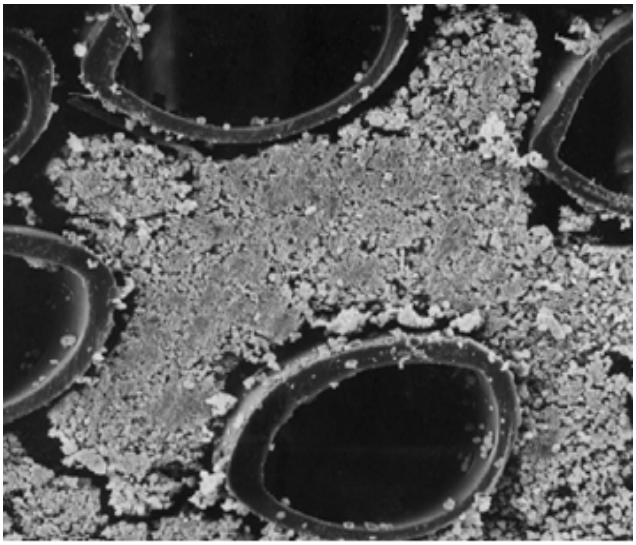
FiberCell Systems Inc. a better way to grow cells

FiberCell 中空纤维细胞培养系统

基因工程和单克隆抗体技术的发展极大地促进了对细胞培养新技术的研究。为了研究出一种简单、经济的细胞培养系统，生物技术的一个新领域诞生了，美国 FiberCell 系统有限公司一直致力于提供简单易用的中空纤维细胞培养系统，让研究人员和生物技术公司 生产不可能使用传统的细胞培养的方法大规模的生产细胞及细胞的产品。

全新的大规模细胞培养方式--Hollow Fiber 细胞培养

中空纤维细胞培养新技术-新工具--可提高 100 倍的产量，节省 20 倍的耗材.



中空纤维是一种很小的、圆柱形的透滤材料，形状类似于喝水用的吸管，并且直径只有人的头发丝般粗细（200 μm）.
人们将大束的纤维装入圆柱形的外壳中，这样，一些自筒末端（末端通道）进入的液体会流过纤维内部，而圆柱形外壳外的侧面通道可以通到纤维的外部区域（毛细管外层空间，或者 E CS）。通常，细胞被放在纤维外，那里它们能够贴壁生长，而细胞培养基可以在纤维内不断地循环以提供细胞所需的营养和氧气。透滤材料的性质决定了像葡萄糖和乳酸盐这样的小分子可以随意地穿过纤维，而蛋白质这样的较大分子则不能穿过。如果有细胞因子或自分泌因子存在（它们可以加强或抑制细胞的生长）， 则可以通过选择纤维的孔径或者截留分子量（ M W C O ）来控制不同因素对细胞生长的影响。

图 1 显示纤维和培养中的淋巴细胞的中空纤维筒横截面。

四川省, 成都市上东大街 36 号, 新和•春禧时代 12-11, 中国, 四川省. 邮编 610016 www.ahmedical.com.
USA: 01-914- 498-4158; 中国 电话及传真: **028-86653817;** 电子邮件信箱: ahmedical@verizon.net;

35 Weaver Street, *Scarsdale*, New York 10583. U.S.A.

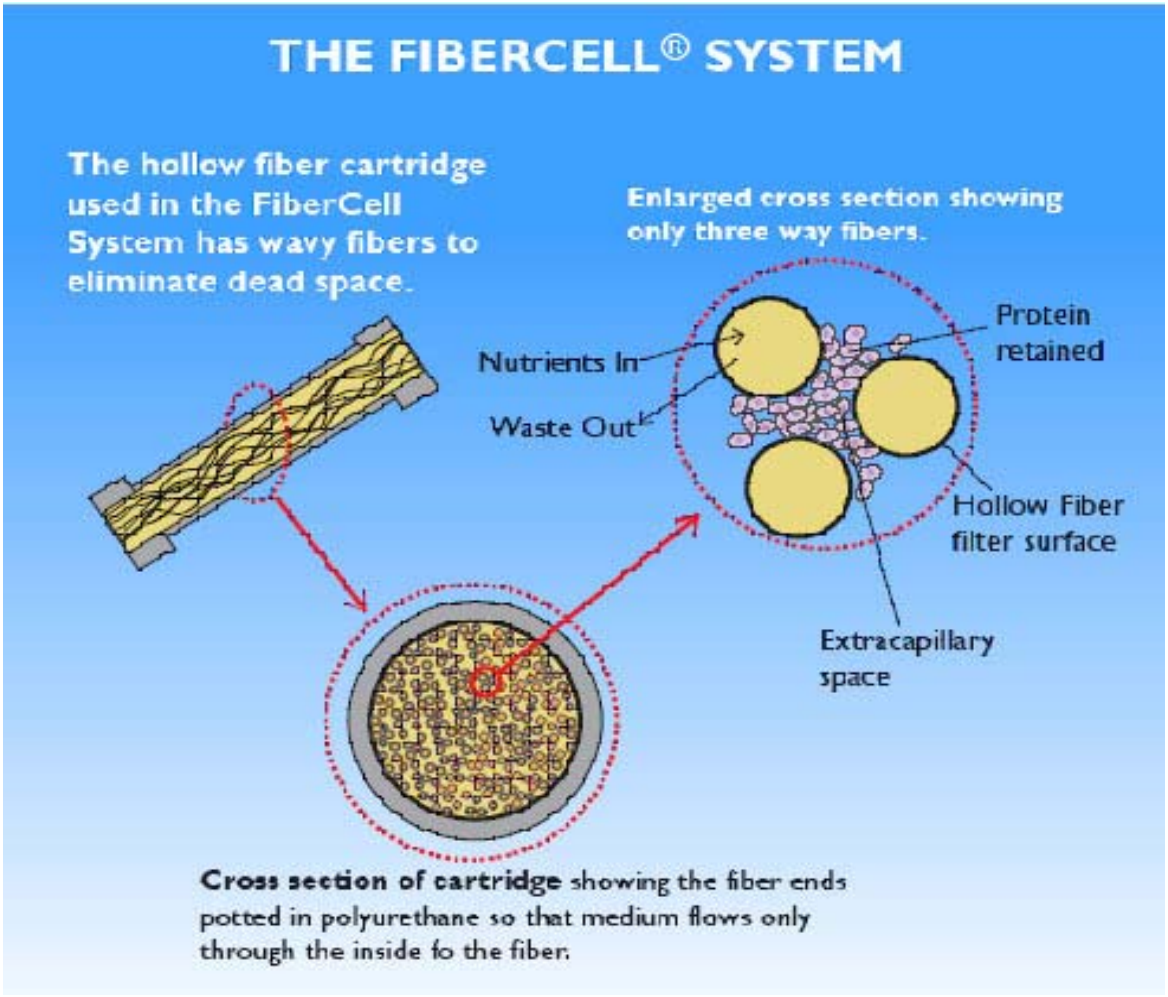


图 2. **FiberCell 中空纤维细胞培养系统** 利用 hollow fiber membrane 隔离细胞与细胞培养液，在中空纤维的外壁（exra-capillary space, ECS）培养细胞的装置。此装置主要為模擬生物體循環系統中毛纤维的结构及功能；由具半透析性之多孔膜狀高分子-- polysulfone 天然亲水聚合物，拉成兩端有開口的纤维。將此種中空纤维裝入柱狀的塑胶容器中，其成品就像光纤排列在电缆中一样。

中空纤维生物反应器的一个特点是培养的细胞浓度可以超过 108/mL。而一般的旋转烧瓶培养的哺乳动物细胞浓度大约是 106/mL。高浓度细胞可以产生高浓度分泌蛋白，并能进行有效地细胞感染，还可以减少细胞对血清的需求甚至使细胞在无血清培养基中生长。中空纤维生物反应器和其他细胞培养技术的另一个基本区别在于：中空纤维能形成易于细胞附着的多孔渗滤支撑，最类似于活体内的细胞生长方式。由于营养输送是由下至上的，因此细胞很容易彼此堆积，形成一个具有多层细胞的层面。在中空纤维生物

35 Weaver Street, *Scarsdale*, New York 10583. U.S.A.

反应器上进行细胞传代是不必要的。根据它们的生长特点，这些培养物可以保留到扩增期。已经有研究人员用单根 FiberCell 中空纤维生物反应器连续一年生产一种单克隆抗体。中国仓鼠卵巢细胞（CHO）株和 HEK293 人胚肾细胞株可以在三个月或者更长时间在同一个生物反应器中进行蛋白表达。

应用

- **单克隆抗体试验生产或量产** Monoclonal Antibodies:
  每月可生产 100 mg-2000 mg 抗体，比用培养瓶生产抗体的浓度高 100 倍，可达到 0.5-5 mg/mL;

- **重组蛋白表达或量产** Recombinant Proteins:
  每天可生产 1-10 mg 重组蛋白，生产分泌蛋白的表达量是培养瓶培养的 100 倍，可达到 100-500 μg/mL;

- **条件培养液和细胞因子的生产** Conditioned Medium & Cytokine Production;

- **内皮细胞/肌细胞培养及形态研究** Endothelial Cells/Muscle Cells:
  在纤维内培养内皮细胞的同时，可在纤维外层培养另一种细胞（如血管平滑肌细胞）。纤维内表面积相当于一个 T75 培养瓶，一个培养筒可提取约 100mg 的 RNA 量。并可通过调整剪切力大小诱导内皮细胞发生形态改变，模拟体内正常与异常血压;

- **可用来检验抗癌、抗病菌（包含 HIV/HBV/HCV）以及抗寄生虫等药物反应、研究及生物测试** Bioassays;

- **病毒扩增**: 运用 Virus Production 可生产超过 $1-3\times10^{13}$ 病毒颗粒，相当于 20 个滚瓶的产量;

- **淋巴细胞扩增培养及研究** Lymphocyte Expansion:
  细胞培养浓度可达 1×108+/mL;

- **体外毒性研究与分析** *In Vitro* Toxicology;

- **病原体培养** Malaria Culture
  一次培养可收获相当于 60 个 T25 培养瓶的产量。

- **人工器官研究** Artificial Organ Research;

- **干细胞培养与其他细胞外基质和细胞因子培养就很重要了。**
  Stem cell and other cultures where extra-cellular matrix and cytokines can be important.

图 3. 一组 C2011 FiberCell System bioreactor（2,100 cm^2 surface area）相当于一天 20 瓶以上的 Roller bottle（800 cm^2 surface area）产量。

系统特点:

1. FiberCell 使用波浪状的 Polysulfone PlusTM 中空纤维，波浪状可确保筒式培养系统内间隙一致，中空结构使其表面及周围皆可供细胞生长，纤维大量表面积还可供养分交换，是附着型与悬浮型细胞最佳培养系统！
2. 专有的正压式可置换蠕动泵系统可延长筒式培养系统的寿命，同时也能加速纤维间养分及代谢物的交换。
3. 代谢物及抑制因子可自细胞中分离出。乳酸和葡萄糖等小分子可轻易地穿过纤维。单株抗体和蛋白等大分子会被保留在毛细管间隙里。
4. 中空纤维可被活化并让重组蛋白、抗体及生长因子结合于其表面，允许长期培养以检测细胞外间质(extra-cellular matrix)的影响及生物活性的研究。
5. 亲水性的 Polysulfone 纤维的过滤率较传统方式（cellulosic fiber）高出 10 倍，可增加细胞的存活率及优化生长状态。
6. 封闭的生物安全系统可避免危险性生物对人体的威胁（P3 实验室适用）。
7. 操作简便、耗材用量少、可降低血清需求量：

完整组装的无菌耗材可立即使用。

- 一天只需几分钟即可简易操作大量的细胞。
- 可进行为期六个月以上的长期细胞培养，而不需更换耗材
- 一组中空纤维细胞培养系统的产量相当于 20 瓶滚瓶产量
- 收取体积小，提高了抗体及蛋白质的浓度，方便纯化。
- 细胞密度高，可快速、一致的研究感染性病毒和其它微生物病原。

35 Weaver Street, *Scarsdale*, New York 10583. U.S.A.

筒式培养系统规格：

型号	大小	表面积	纤维质型号	包装密度	ECS 容积	MWCO 50%	MWCO 95%	细胞最大培养量
C2025	小	75 cm^2	Activated PS	30%	1.5 mL	0.1 μm	0.1 μm	10^8
C2008	中	2100 cm^2	low flux PS	50%	15 mL	5 kd	20 kd	10^9
C2011	中	2100 cm^2	high flux PS	50%	15 mL	20 kd	100 kd	10^9
C2003	大	1.2 m^2	low flux PS	50%	70 mL	5 kd	20 kd	5×10^{10}
C2018	大	1.2 m^2	high flux PS	50%	70 mL	20 kd	100 kd	5×10^{10}
C2019	大	1.2 m^2	high flux PS	50%	70 mL	20 kd	100 kd	5×10^{10}
C4005	加大	2.5 m^2	low flux PS	50%	150 mL	5 kd	20 kd	10^{11}
C4020	加大	2.5 m^2	high flux PS	50%	150 mL	20 kd	100 kd	10^{11}

SMALL Cartridge
4300-C2025 0.1μm pore size for the highest exchange rates. Activated fiber for attachment of matrix proteins, cytokines and antibodies. Ideal fiber for endothelial cell and hepatocyte culture.

MEDIUM Cartridges
4300-C2008 Low MWCO (5kd@50%) hydrophilic fiber for trapping smaller molecules. Suggested for recombinant proteins between 25kd and 100kd. Appropriate for suspension cell lines including CHO, HeLa and 293. Can support up to 10 9 cells and produce 100μg/mL of recombinant protein in 15mL ECS.

4300-C2011 High MWCO (20kd@50%) hydrophilic fiber for trapping larger molecules, hybridoma culture and lymphocyte culture. 20kd MWCO allows TGF beta and TNF alpha to diffuse away while retaining antibodies. Can support up to 10 9 cells and produce 5-50mg of monoclonal antibody every two days. Also used for medium scale adenovirus production at levels of 1- 5 x 10 12 pfu (plaque forming units).

LARGE Cartridges
4300-C2003 Low MWCO (5kd@50%) hydrophilic fiber for trapping smaller molecules. Suggested for recombinant proteins between 25kd - 100kd.

4300-C2018 High MWCO (20kd@50%) hydrophilic fiber for trapping larger molecules, hybridoma and lymphocyte cultures. 20kd MWCO allows TGF beta and TNF alpha to diffuse away while retaining antibodies. Can support up to 10 11 cells and produce 75-150mg of monoclonal antibody every two days. Appropriate for adherent suspension cell lines including CHO, HeLa and 293 cells. Can support up to 10 11 cells and produce 100μg/mL of recombinant protein in 70mL ECS.

X-LARGE Cartridges

35 Weaver Street, *Scarsdale*, New York 10583. U.S.A.

4300-C4005 The C4005 cartridge is intended for use in larger hollow fiber cell culture systems from other manufacturers and does not include a flow path stand or oxygenator tubing. Side ports have 3" of tubing capped with luer fittings, end ports are 3/8" hose barbs. High gross filtration rate and polysulfone fiber are superior to cellulose acetate for recombinant protein production.

4300-C4020 The C4020 cartridge is intended for use in larger hollow fiber cell culture systems from other manufacturers and does not include a flow path stand or oxygenator tubing. Side ports have 3" of tubing capped with luer fittings, end ports are 3/8" hose barbs with tubing and standard luer connectors on the ends. High gross filtration rate and polysulfone fiber are superior to cellulose acetate for recombinant protein and monoclonal antibody production.

What cell types have been cultured in the FiberCell Hollow Fiber bioreactor system.

Essentially if the cells can be grown in flask or other conventional systems then they will grow in a hollow fiber system. It is dependent upon your research and production goals as to whether the cells will behave in the desired fashion. The most common cells types successfully used are:

- Hybridoma cell lines of all species including NSO.
- HEK 293 both suspension and adherent
- CHO
- SP2
- HELA
- Hepatocytes
- Cancer cell lines
- COS cells
- HIV and B lymphocytes

- And many others.

What are the advantages of hollow fiber cell culture?

Hollow fiber bioreactors support cells at 10-100X higher density than regular cell culture methods. This means the cells are in a more *in vivo* like environment and require less serum, can be more easily adapted to a serum free medium or can be supported with a simplified serum replacement like CDM HD.

Secreted products will be concentrated by the filter-like behavior of the hollow fibers, typically 100X higher concentration than that found with traditional bioreactors.

The effects of cytokines such as TGF-Beta or TNF Alpha can be controlled by the selection of the pore size of the fiber.

Hollow fiber bioreactors permit the culture and handling of large numbers of cells in a way that might not be practical using other methods in most laboratories.

Cells are bound to a porous support so they are free to grow in a post confluent fashion. Cells do not need to be split and can grow for extended periods of time. Hybridomas will typically produce antibody for 6 months or longer, CHO and 293 for 3-6 months of continuous production. The record is 2 years of continuous growth of a glioma cell line.

How does the pump work?

The FiberCell Duet pump uses a positive pressure displacement method that incorporates two one way check valves to drive the medium through the cartridge. This ensures reliable flow and long cartridge life.

How is gas controlled?

There is a loop of silicone tubing wrapped around the core of the cartridge flow path stand. Silicone tubing is very gas permeable and the gas composition of the medium will be the same as the gas composition in the incubator.

How is temperature controlled?

The Duet pump is designed to fit inside a standard CO2 incubator, the thin cord is designed to fit through the glass door.

What flow rate should I use?

The rate limiting factor in hollow fiber cell culture is the low partial pressure of oxygen due

to lower solubility at 37 degrees. For this reason generally flow rate should be at the higher levels, between 26-30 on the Duet Control Box for the larger cartridges (C2003 and C2018) and between 22-26 for the medium sized cartridges. At the initiation of culture you will want to use a somewhat lower flow rate in order to allow cytokines to concentrate around the cells.

I want to produce a monoclonal antibody. What cartridge should I use and how much monoclonal antibody can I produce?

Cat #C2011 or C5011 should be used. This MWCO allows TGF Beta to diffuse away while retaining the produced antibody.

C2011 will support up to 1-2 X10 9 cells, this is equal to a one-liter culture or more.

- Produce 5-50mg of antibody every 2 days, average is 20mgs per harvest
- Continue to produce antibody for up to 6 months of continuous culture
- Consumes about 1 liter of cell culture medium every two days. To reduce medium consumption harvest more cells out.
- A single mouse used for ascites fluid production will produce 10-20mgs total antibody; each harvest is equal to a single mouse.
- FiberCell cartridge has about 4-5 times the production capacity of the CellLine flask per harvest.
- Endotoxin burden is 1/10 th that of ascites fluid production.
- Cannot be re-used but can be stored and re-inoculated with the same cell line. product in the supernatant will be your antibody, simplifying your purification.
- Hybridomas grown in the FiberCell cartridge can be more easily adapted to serum free cell culture medium or adapted to as low as 2% FBS. When CDM HD is used the only

C5011 Will support up to 2-4 X10 10 cells ; this is equal to a two liter culture.

- Produce 10-100 mgs of antibody every 2 days, average is 40mgs per harvest
- Consumes about 2 liters of cell culture medium every day.
- Scale up cartridge from C2011.
- Endotoxin burden is 1/10 th that of ascites fluid production.

I want to produce a recombinant protein. What cells can I use and what sort of production can I expect?

Hollow fiber bioreactors can be used with any cell type that will grow in flask or spinner culture. Stable transfectants should be used to take full advantage of the long term production potential offered. CHO, 293, HEP G2 and many other cell types have been used. Insect cell culture is not ideal due to the transient nature of the culture but constituitive

expression in S2 cells have had excellent results at lower induction agent concentrations

Recombinant Protein Production

The molecular weight of the protein to be produced determines the fiber MWCO to use.

For proteins larger than 100kd use 4300-C2011 or 4300-C2018

For proteins of 20kd to 100kd use 4300-C2008 or 4300-C2003

Production will be typically 100X that of flasks with harvested product concentrations between 100 micrograms and 300 micrograms per ml per day.

4300-C2011 Has 2200cm 2 of surface area, equal to 12.5 T175 flasks. Because of the way cells grow when attached to a fiber the total number of cells will be equal to 50-60 T175 flasks.

- If the protein of interest can be trapped by the molecular weight cut-off of the fiber it will reach a concentration 100 times that of the same cell line grown in flask culture when collected from the ECS (extra-capillary space)
- Average productivity is around 100µg/ml (of ECS volume) or up to 1mg per day.
- Proteins greater than 100kd in molecular weight will be trapped by this fiber.
- If the protein of interest is too small to be trapped (like cytokines and cell growth factors) it will reach a concentration of 10 times that of the same cell line grown in flask culture when collected from the reservoir bottle.
- Harvest every day instead of every two days
- Medium consumption same as for hybridomas.
- Reduction in serum concentration or easier adaptation to serum-free medium makes purification of proteins or identification of low concentration growth factors easier.

4300-C2018 Has 1.2m 2 of surface area. This is equal to 68 T175 flasks. Because of the way that cells grow on hollow fibers this will be equal to over 400 T175 flasks.

- Medium consumption will be as high as 4 liters per day
- Can harvest 5-10 mg of protein per day

What medium should I use and should I use serum?

Any cell culture medium that is used in flasks can be used with a hollow fiber bioreactor. However, there are some special considerations to keep in mind.

- The high cell density allows the reduction of the amount of serum to 2% and can

facilitate the adaptation to serum free mediums. The also means that cells can be supported using a simplified replacement for serum such as CDM HD offered by FiberCell Systems.

- Protein free mediums, such as CDM HD, can be used but keep in mind that protein free mediums generally do not contain any attachment factors. Generally we want the cells to attach to the fiber so it is preferred to seed the cells in the presence of serum and then adapt to serum free medium or CDM HD once the cells have reached high density (i.e. consuming 1 gram or more of glucose per day)
- Generally you want to avoid a medium such as RPMI due to the low (2.5 grams per liter) concentration of glucose. This simply means that you would need to change the medium more often, an inconvenience.
- Cells are growing in a stable, shear free environment. The use of surfactants such as pluronic F60 is not required. CDM HD does not contain any surfactants or other membrane protectants.

Should I use antibiotics?

Unless you have a compelling reason to not use antibiotics in your culture medium FiberCell Systems recommends the use of standard concentrations of antibiotics. We can fix anything, as long as you don't contaminate the cartridge. Antibiotics can help prevent the occasional lapse in technique from spoiling a culture. If your concern is endotoxin, and you wish for the absolute lowest levels of endotoxin then it is recommended that you work without antibiotics. Antibiotics can shield an infection but permit endotoxin to accumulate.

What other equipment do I need?

The only other piece of equipment that may not be part of a standard cell culture laboratory would be a way to measure glucose. There are some fancy and expensive machines to measure glucose out on the market but a simple glucometer like the ones used by diabetics and available at just about any drugstore or pharmacy will do the trick. Keep in mind that their readings won't be that accurate above 3.5 grams per liter so it is important to know the starting glucose concentration of your medium.

What are the advantages of hollow fiber cell culture for endothelial cells?

For endothelial cell culture under chronic shear use C2025. This cartridge allows extra-cellular matrix proteins to be attached to the fiber. One of the few ways to grow endothelial cells (cells that line the interior of blood vessels) under conditions of medium flowing over them (like they experience in the body). When grown under these conditions they behave in a much more in vivo like manner. They lay down flat and form a monolayer, form tight junctions, and certain genes are turned on in response to this shear stress that are not expressed in static culture. Other cell types such as vascular smooth

muscle and brain glial cells can be co-cultivated with these endothelial cells.

C4300-2025 will yield about 100µg of total RNA for gene expression analysis.

What are the advantages of using the hollow fiber system for in vitro toxicology?

Hollow fiber bioreactor cartridges from FiberCell Systems offer a simple way to set up a two-compartment model for *in vitro* toxicology with higher levels of reproducible control to complex growth, infection, treatment, and sampling regimens. This system permits more realistic simulation of in vivo drug effects in a dynamically controlled system providing data that more accurately reflects biological responses. The design is fully disposable and will take into account the potential use of weaponized pathogens and genetically modified organisms

What organisms have been used with this system?

- Bacteria including tuberculosis, anthrax, plague, and MDR staph aureus to name a few.
- Viruses including HIV
- Tumor cell lines including breast cancer.

What drugs have been used?

Any type of therapeutic compound can be used. FiberCell offers two fiber types, polysulfone and cellulosic. The polysulfone is preferred because the flux rate and therefore equilibration of drug across the fiber is quite rapid and the geometry of the fibers results in even distribution of the fiber bundle inside the housing. Cellulosic fibers are generally used when the compound to be tested is highly non-polar which can result in significant non-specific binding of the compound to the fiber. Cellulosic fibers will have much lower non-specific binding but also lower flux rates so somewhat slower equilibration times across the fiber.

Advantages of the hollow fiber system:

- Closed bio-safe system
- Organism load can be high enough to match human infections. A high starting number is required to uncover the emergence of drug resistance.
- Drug pharmacokinetics can be exactly modeled on human profile
- Many experiments can be run simultaneously
- Complex systems such as two drug or two cell type cultures can be easily set up.

中空纤维细胞培养的新进展

John J.S.Cadwell

摘要: 基因工程和单克隆抗体技术的发展极大地促进了对细胞培养新技术的研究。为了研究出一种简单、经济的细胞培养系统,生物技术的一个新领域诞生了: 使用生物反应器大规模培养细胞。在某些实验室,基于中空纤维的生物反应器被证明是一种进行细胞培养的理想方法。本文阐述了中空纤维系统的应用研究及其最新进展。

随着基因工程和单克隆抗体技术的发展，细胞培养展现出全新的生命力。经过遗传工程改造的哺乳动物细胞能表达并分泌有科学和治疗价值的蛋白。单克隆抗体技术（是一种将免疫的鼠细胞与无限生长分裂的癌细胞融合在一起的技术）使从培养细胞中得到大量单克隆抗体成为可能。为了大量生产这些可用于商业目的的蛋白，提高细胞培养效率就显得尤为重要。

许多高投入和设备密集型方法都可以规模化生产出所需的蛋白质。随着这些先进的分子生物学技术逐渐步入到普通的实验室中，一些研究生已可以制备出重组蛋白或单克隆抗体。对于学院式研究或是刚起步的生物技术公司来说，利用大制药公司所使用的设备进行生产是不切实际的。为了研究出一种简单、经济的细胞培养系统，生物技术的一个新领域诞生了：利用生物反应器进行大规模细胞培养。在实验室规模的研究中，基于中空纤维的生物反应器已被证明是一种培养 10^9–5×10^{10} 个细胞的理想方法。本文阐述了中空纤维系统的应用研究及其新进展。

中空纤维是一种很小的、圆柱形的透滤材料，形状类似于喝水用的吸管，并且直径只有人的头发丝般粗细（200 μm）（见图 1）。人们将大束的纤维装入圆柱形的外壳中，这样，一些自筒末端（末端通道）进入的液体会流过纤维内部，而圆柱形外壳外的侧面通道可以通到纤维的外部区域（毛细管外层空间，或者 ECS）。通常，细胞被放在纤维外，那里它们能够贴壁生长，而细胞培养基可以在纤维内不断地循环以提供细胞所需的营养和氧气。透滤材料的性质决定了像葡萄糖和乳酸盐这样的小分子可以随意地穿过纤维，而蛋白质这样的较大分子则不能穿过。如果有细胞因子或自分泌因子存在（它们可以加强或抑制细胞的生长），则可以通过选择纤维的孔径或者截留分子量（MWCO）

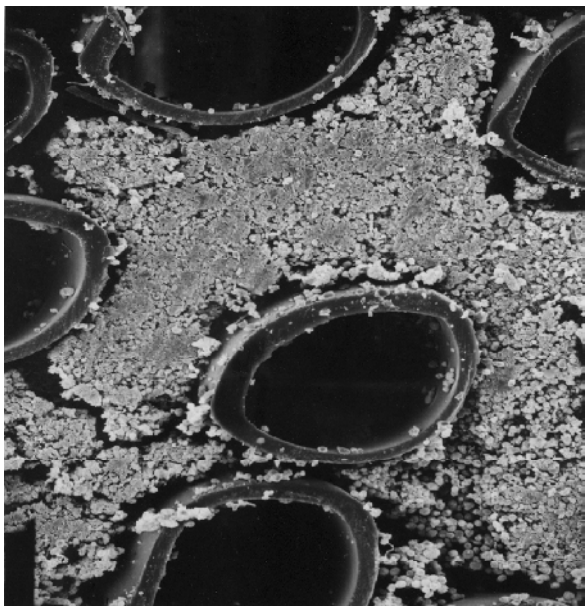


图1 显示纤维和培养中的淋巴细胞的中空纤维筒横截面。

作者简介: Mr. S. Cadwell is President, FiberCell Systems, 905 West 7th St. #334, Frederick, MD 21701, U.S.A.; tel.:301–471–1269; fax: 301–865–6375; e–mail: jcadwell@fibercellsystems.com

（molecular weight cutoff）来控制不同因素对细胞生长的影响。

Knazek 等[1]首次将中空纤维系统用于细胞培养，其发展的细胞培养方法同一些体外方法一样，可以得到 10^8cells/mL 或者更多，而不是只有 10^6/mL（利用标准细胞培养技术得到的细胞量）。中空纤维系统可以在很小的体积内提供非常大的表面积，其表面积可以达到 200cm²/ml，从而可以在一个非常小的体积范围内让大量细胞附着。细胞通过纤维壁可以十分有效地交换营养和代谢物，并且由于在制造过程中可以控制纤维的 MWCO，从而可以调整纤维的过滤性能，使之保留纤维特异蛋白质和细胞因子或者允许它们通过纤维进入循环基质当中。

尽管中空纤维生物反应器并不是一项新技术，但是随着材料和方法的改进，这一技术得到了不断地创新。下面是两个典型的实例。第一个例子是一种新型的聚砜材料，这种材料可以提供极高的总滤过率——一个有 30-kD MWCO 的典型纤维质纤维的总滤过率大约是 15（mL/min/cm²/mm Hg/hr）。FiberCell 20-KD MWCO 的聚砜纤维(catalog nos. C2011 and C2018, **FiberCell Systems**, Frederick, MD)的总滤过率超过 140.2。另外一个例子是“波”的应用。波被引入到在外壳内均匀分布的纤维束中，由于波的作用使得纤维束中的所有纤维具有一致的性能，从而消除了沟道效应（channeling effect）和由纤维间隔过大而引起的死区。

体积只有 15mL 的中号中空纤维生物反应器（catalog nos. C2011 and C2008, **FiberCell Systems**）能提供2200cm²的表面积。体积为60ml的较大的反应器（C2018 和 C2003）可以提供 1.2 m² 的表面积。

中空纤维生物反应器的一个特点是培养的细胞浓度可以超过 10^8/mL。而一般的旋转烧瓶培养的哺乳动物细胞浓度大约是 10^6/mL。高浓度细胞可以产生高浓度分泌蛋白，并能进行有效地细胞感染，还可以减少细胞对血清的需求甚至使细胞在无血清培养基中生长。

中空纤维生物反应器和其他细胞培养技术的另一个基本区别在于：中空纤维能形成易于细胞附着的多孔渗滤支撑，最类似于活体内的细胞生长方式。由于营养输送是由下至上的，因此细胞很容易彼此堆积，形成一个具有多层细胞的层面。在中空纤维生物反应器上进行细胞传代是不必要的。根据它们的生长特点，这些培养物可以保留到扩增期。已经有研究人员用单根 FiberCell 中空纤维生物反应器连续一年生产一种单克隆抗体。中国仓鼠卵巢细胞（CHO）株和 HEK293 人胚肾细胞株可以在三个月或者更长时间在同一个生物反应器中进行蛋白表达。

1 单克隆抗体的生产

中空纤维生物反应器的第三个特点是可以通过细胞因子的分子量来调节它们对细胞生长的影响。最常见的例子是用杂交瘤细胞株生产单克隆抗体。这是中空纤维生物反应器的第一次大规模应用。杂交瘤细胞分泌组织生长因子 - β (TGF- β)，它是一种可以抑制杂交瘤细胞生长的细胞因子。功能型 TGF- β 的分子量大约是 27kD。一根有着合适 MWCO 的中空纤维过滤器允许 TGF- β 扩散通过，而杂交瘤细胞分泌的抗体则在毛细管外层的空隙间堆积，并达到很高的浓度。通过稀释细胞因子浓度从而使之进入循环介质可以减小或者消除 TGF- β 的抑制效果。这一机理对淋巴细胞同样有效；淋巴细胞中有抑制其生长的肿瘤坏死因子 - α (TNF- α)。

采用中空纤维生物反应器生产单克隆抗体的优点包括：

——比用烧瓶培养生产抗体的浓度高 100 倍，可以达到 0.5-5mg/mL。

——收集液体积为15-60mL，每两天收集一次。

——利用杂交瘤细胞适合在无血清培养基中生长的特性易于得到高细胞浓度（见图2）。

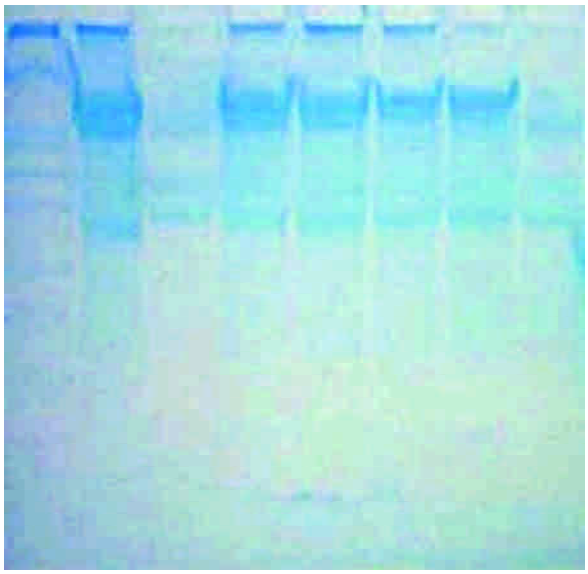


图2 循环着的无血清培养基和由毛细管外层空间获得的几组收集液的凝胶结果。

道A：MW标准品。道B，D，E，F和G：从毛细管外层空间得到的收集液。道C：循环着的无血清培养基。凝胶结果显示，分子量为147kD的IgG保留在纤维上，而分子量为60kD或者更小的分子则能过穿过纤维进入循环基质中。

——与通过腹水产生的单克隆抗体相比，用中空纤维生物反应器生产的单克隆抗体可以减少内毒素的产生。

——利用中空纤维生物反应器可以很容易地得到未知的、人源的和非鼠源的抗体。

——利用中空纤维生物反应器可以连续六个月（或是更长时间）不停地生产抗体。

一个中空纤维生物反应器系统能够生产100mg到几克抗体。

2 分泌蛋白的生产

人们热衷于中空纤维生物反应器的另一个原因是它可以用于重组蛋白质的生产。CHO和HEK293细胞株最长采用的细胞株，但人们也用其它类型的细胞株进行重组蛋白的生产，包括一些昆虫细胞株（例如：果蝇细胞）。假如没有抑制性细胞因子，纤维MWCO的选择就仅仅取决于分泌蛋白的分子量大小。用中空纤维生物反应器生产分泌蛋白的表达量是烧瓶培养的100倍，可以达到100-500 μg/mL。

图3给出了在无血清培养基中表达重组IgG蛋白的HEK293细胞株表达的蛋白质数据和分泌的乳酸盐数据。通常，当葡萄糖剩至一半时就要更换培养基，但周末除外。在周五下午，要收集大量的产物以减少细胞量，仅向系统中加入1L新鲜的培养基就能使之度过整个周末。典型的系统每两天消耗1L无血清培养基。有两个现象非常有趣。首先是生产效率，尽管每天的产量都有显著变化，但在一段时间内，如果收集液体积为20mL，得到的蛋白产量都接近800 μg/mL。总体上说，900mL体积培养2个月后可以得到276mg蛋白质，平均浓度为300 μg/mL，每天4.5mg。

另一个有趣的现象很容易从图中见到。乳酸盐浓度和蛋白质产量在周一达到峰值。此时，已经有两天多没换过培养基了并且也没

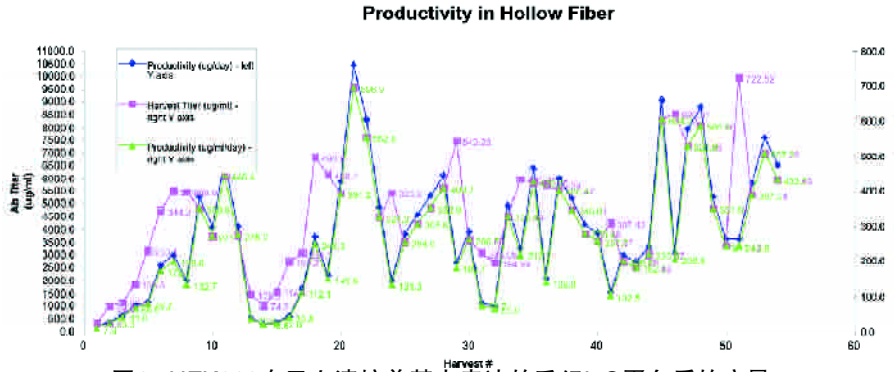


图3　HEK293在无血清培养基中表达的重组IgG蛋白质的产量。

有收集过产物。蛋白质有机会在周末得以聚积，而培养基则被完全消耗。葡萄糖低于100mg/mL；pH低于6；有大量的乳酸盐产生，最大值为3.8mg/mL。尽管一两天以后细胞的代谢率显著降低，但令人吃惊的是，在这种乳酸盐浓度下仍有大量细胞存活并不断表达产物。随着培养物的成熟和细胞浓度的增加，恢复时间变得更短。在FiberCell盒中生长的其它类

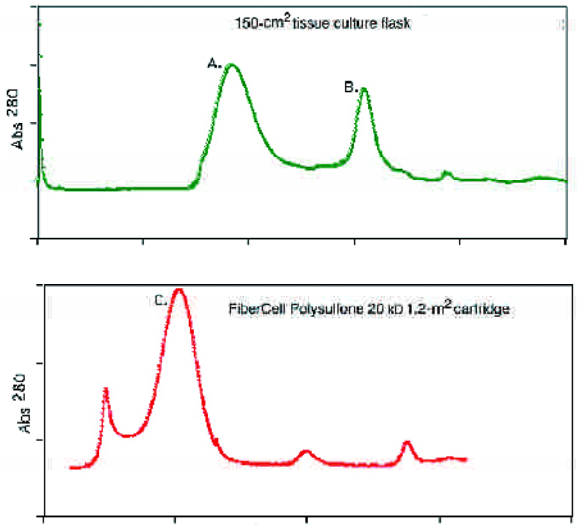


图4 对用烧瓶培养生产的hexerimized IgG 1和用FiberCell中空纤维生物反应器培养生产的hexerimized IgG 1进行比较。

型细胞也可以观察到这一现象，这种对乳酸盐的高抗性是纤维总滤过率高的结果。

图4对在T150烧瓶和在FiberCell中空纤维生物反应器系统中进行培养的重组CHO细胞株的蛋白产量进行了比较。重组CHO细胞在T150烧瓶中培养至将要汇集成片时进行收集并接种到生物反应器中。它表达的蛋白是hexeramized IgG1，由6个带有3个IgA尾的IgG1亚基组成的。在烧瓶中，大约有40%的表达蛋白以没有进行适当折叠的单体亚基形式存在；而转入FiberCell生物反应器后，大约有95%的蛋白以正确折叠的六聚形式存在。总体积4L的体系10周可以产生475mg蛋白。用中空纤维系统生产重组蛋白质的优点和用其生产抗体的优点相同，此外，非常复杂和/或高度糖基化的蛋白质通过改进蛋白折叠方式与烧瓶培养产生的同种蛋白相当。中空纤维生物反应器可以生产10mg到几百毫克的重组蛋白，这是一种非常有用的手段。

3　病毒的生产

应用中空纤维生物反应器生产病毒对研究人员具有很大吸引力。高浓度生长的细胞为病毒繁殖提供快速、一致的感染动力学基础，也能够获得具有很高滴度的病毒。对于不同的病毒，有效的生产方法也可能有所不同。许多生产病毒的初步尝试都是用3T3或者PA317包装的细胞株生产逆转录酶病毒。这些细胞株源于纤维化组织的成纤维细胞，非常易于贴壁附着。细胞很快就能长满中空纤维盒，使收集液体积由10–15mL减少到只有0.5mL。尽管可以得到高滴度的病毒，但是由于重新获得的病毒总体积很小，使得这一方法效率很低。此外，如果必须以感染的方式繁殖病毒，那么只有外层细胞可能会被感染而产生病毒。

某些改良过的用于细胞株悬浮培养的无血清培养基的出现解决了中空纤维系统生产病毒的这一缺陷。如果HEK293细胞能够适应悬浮培养，那么就可以用腺病毒或其它需要感染才能繁殖的病毒对其进行感染从而产生病毒。这一方法的最早由Chung等[2]报道的，该方法也在阿拉巴马州基因治疗中心（伯明翰）Alex Kotov的努力下得到发展[3]。其基本原则是：如果包装细胞可以进行悬浮培养，并且病毒可以通过感染转入细胞内，那么就可以用中空纤维系统大量生产该病毒。

已经应用这种方法成功地生产出爱泼斯坦－巴尔病毒和HIV病毒。有时可以看到，用中空纤维系统生产的HIV病毒比用烧瓶培养生

产的多出1000倍。对于中空纤维生物反应器系统来说，生产病毒是一个相对较新的应用并且仍在进一步发展中。

4　疟原虫培养

培养疟原虫需要感染红血球细胞，即费力又耗时。疟疾寄生虫是相对厌氧的生物体，并且利用葡萄糖的能力很差。这意味着培养基需要不断更换。在烧瓶中生长的红血球细胞浓度通常要求不超过6%的血球比率。健康国立研究所（Bethesda,MD）[4]采用一种改进的方法在FiberCell盒中培养红血球细胞使之浓度达到100%血球比率，并且可以用疟原虫在盒内直接进行感染。一盒收集量相当于60多个T25烧瓶的产量。

5　改进型聚砜（PS+）纤维

抗体的生产，蛋白的表达和条件培养基（conditioned medium）的传代是中空纤维生物反应器的一些传统应用。中空纤维生物反应器的更多应用不再是利用它们极大的细胞容量，而是利用它们的其它能力。改进型聚砜（PS+）纤维（FiberCell系统）表面易于吸附蛋白质，细胞因子、抗体，或其它类蛋白物质。这种纤维可以用70%乙醇进行活化，活化的纤维吸附蛋白质的浓度可以达到10–100ug/cm^2。这种纤维可以用于研究生物化学表面对特殊细胞长期培养的效果。

6　内皮细胞培养

标准培养瓶中培养的内皮细胞生长活跃并不断分裂，但它们并不形成紧密连接。在持续切应力和充足培养基下生长的内皮细胞会以生理方式产生作用。以此方式生长的内皮细胞能形成单层、饱和的紧密型连接。基因表达谱和蛋白表达谱一样受到影响[5–7]。由Barbara Ballerman博士、Eudora Eng博士最初还有霍普金斯大学（巴尔的摩，MD）的Johns提供的尚未发表的数据表明，血管内皮生长因子（VEGF）可以引起低切变条件下生长的内皮细胞增殖率的增加。

不同大小的切应力可以诱导内皮细胞发生形态改变。图5对比了人肺内皮细胞在低切变（5 dynes/cm^2）和高切变(15 dynes/cm^2)下的不同效果。低切变照片（图6）中可以看到在纤维表面有一单层细胞。而在高切应力下，细胞则以丛状损伤的方式堆积在一起（图7）。在体内也可以观察到这一现象，而在烧瓶中培养时则观察不到。

如图8所示，在纤维内培养内皮细胞的同时，可以在纤维外层培养另一种细胞。纤维内培养内皮细胞，纤维外培养血管平滑肌细胞时，试验表明，当流动速律变化时，平滑肌G–蛋白结构和内皮素受体表达会受到直接的影响。原因是由内皮细胞分泌的某些能够穿过纤维的物质可以使平滑肌细胞发生改变。

改进型聚砜纤维可以用来研究细胞外基质

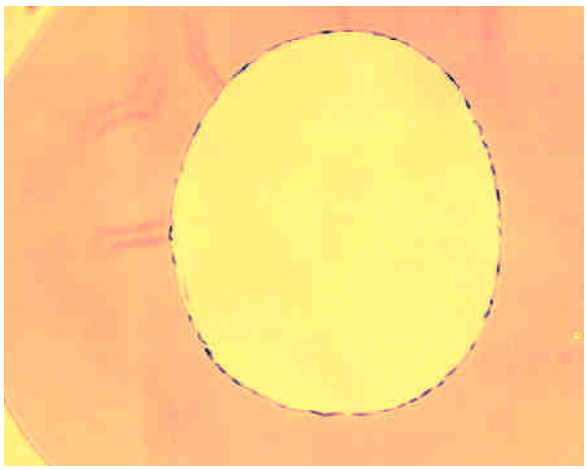


图5　内部接种有脐静脉内皮细胞（HUVECs）的纤维横截面。

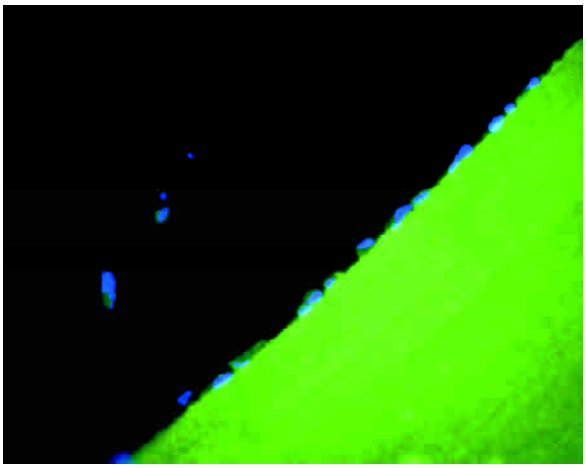


图6　生理性切应力(5 dynes/cm²)下的肺内皮细胞。

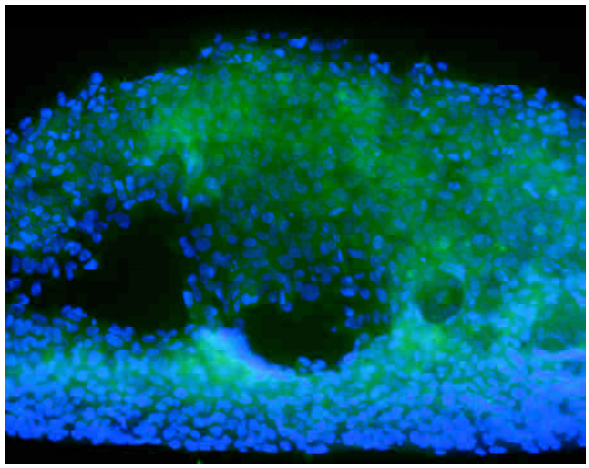


图7　病理性切应力(15 dynes/cm²)下的肺内皮细胞。

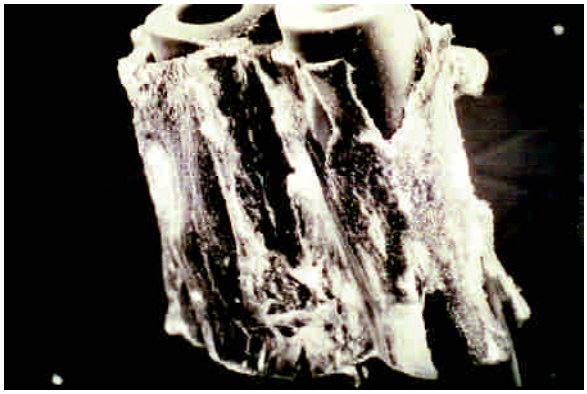


图8 与血管平滑肌共同培养的牛大动脉内皮细胞。

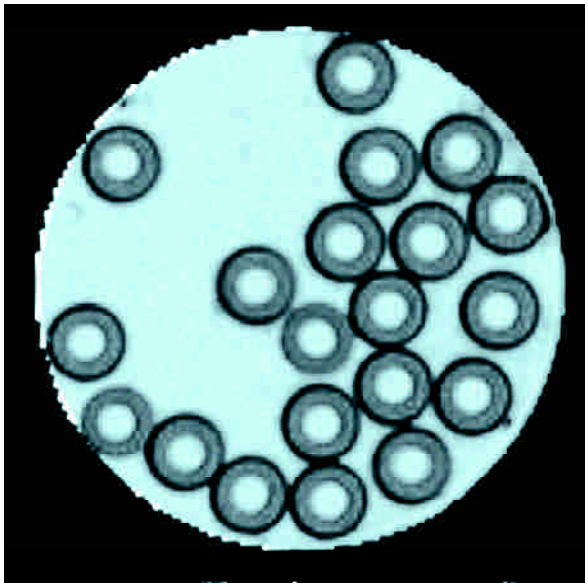


图9 在包被有胎牛血清的纤维表面上生长的成骨细胞。

对不同细胞长期培养的影响。图 9 显示了生长在涂有血清蛋白的纤维表面的成骨细胞。由于图中的核磁共振图像分辨率相对较低，所以这些细胞本身不能被清楚地分辨。然而，围绕纤维的黑圈表明有无机物沉淀存在，这是形成骨骼的第一步。通过这种方法也可以研究其它类型的基质和骨形态发生肽（bone morphogenic peptide (BMP-2)）的效果。

改进型聚砜纤维还可以用于附着能刺激淋巴细胞增生的特异抗体和促进肝细胞或胰岛细胞生长的特异配体，并且可以用来研究细胞外基质对细胞长期生长和分化的影响。用这种纤维可以研究细胞的生长和分化，而以前传统的细胞培养系统则不能实现。

尽管这并不是一项新技术，但是纤维材料的显著优点导致了中空纤维生物反应器系统的产生，这一系统即提高了生产效率，又简化了操作过程。应用中空纤维生物反应器能够生产 100 毫克到几克的抗体或者重组蛋白质，也可以在一些研究室里培养 10^9-5 × 10^{10} 个细胞。用这一系统培养和生产病毒的操作方法仍在不断发展之中，这使它能生产更大浓度的病毒产物的优点显得更为突出。或许 30 年后，中空纤维生物反应器仍是用于实验室大

规模细胞培养的首选方法。

参考文献

1. Knazek RA, Gullino PM, Kohler PO, Dedrick RL. Cell culture on artificiall capillaries: an approach to tissue growth in vitro. Science 1972; 178(56):65–6.
2. Gardner TA, KoSC, Yang L, Cadwell JJS, Chung LWK, Kao C. Serum–free recombinant production of adenovirus using a hollow fiber capillary system. BioTechniques 2001; 30(2):422–8.
3. Isayeva T, Kotova O, Krasnykh V, Kotov A. Advanced methods of adenovirus vector production for human gene therapy: roller bottles, microcarriers, and hollow fibers. BioProcessing J2003; 2(4):75–81.
4. Li T, Glushakova S, Zimmerberg J, A new method for culturing Plasmodium falciparum shows replication at the highest erythrocyte densityes. J Infect Dis 2003; 187:159–62.
5. Ott MJ, Ballermann BJ. Shear stresss–conditioned, endothelial cell–seeded vascular grafts: improved cell adherence in response to in vitro shear stresss. Surgery 1995; 117(3):334–9.
6. Waybill PN, Chinchilli VM, Ballermann BJ. Smooth muscle cell proliferation in response to co–culture with venous and arterial endothelial cells. J Vasc Interv Radiol 1997; 8(3):375–81.
7. Redmond E, Cahill P, Sitzman J. Flow mediated regulation of endothelin receptors in co–cultured vascular smooth muscle cells: an endothelium–dependent effect. J Vasc Res 1997:895–902.

New Developments in Hollow–Fiber Cell Culture

John J.S. Cadwell

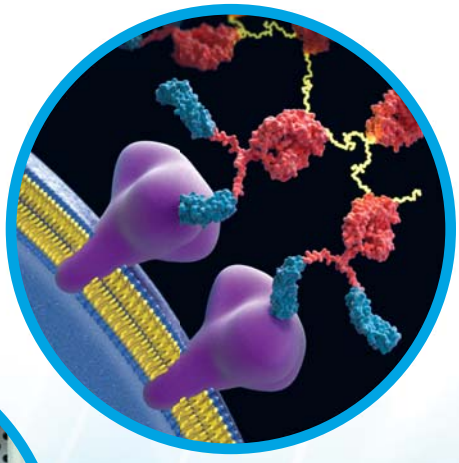
Abstract: The growth of genetic engineering and hybridoma technologies has been a strong impetus for the investigation of new techniques for cell culture. The search for a simple, cost–effective cell culture system has resulted in the birth of a new area of biotechnology: the use of bioreactors to grow cells on a large scale. A bioreactor based on hollow–fiber bundles is shown to be an ideal method for culturing cells in any laboratory. Classical applications of hollow–fiber systems are described, in addition to some more recent ones.

（赵鹏译，张维冰校）

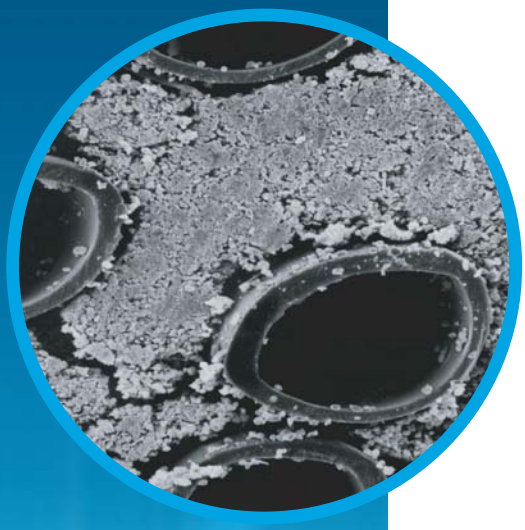
HOLLOW FIBER BIOREACTORS
AND RELATED PRODUCTS

Designed for the following applications:
Monoclonal Antibody Production
Recombinant Protein Production
Endothelial Cell Culture
Lymphocyte Culture and Expansion
In Vitro Toxicology

FiberCell Systems Inc.
a better way to grow cells



www.fibercellsystems.com



ABOVE: Cross-section of hollow fibers in FiberCell® Systems hollow fiber bio-reactor. High-density cell growth is shown in extra-capillary space.

- Cells grow on and around hollow fibers.
- Fiber geometry is optimized for both adherent and suspension cell types.
- Small molecules such as lactate and glucose can easily cross the fiber.
- Large molecules such as monoclonal antibodies and proteins are retained and concentrated in the small volume of the extra capillary space.

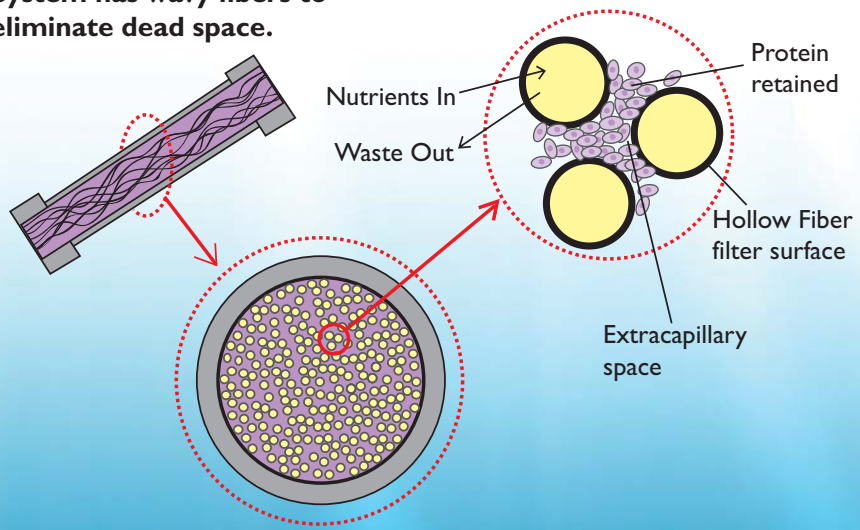
What is the FiberCell® System advantage?

- Provides 10X the gross filtration rate of cellulosic hollow fibers.
- Improves cell viability.
- Eliminates dead spots with uniform fiber spacing and "wavy" fibers within the cartridge.
- Dialyzes waste and inhibitory cytokines away from cells.
- Stabilizes the PH of the cell culture medium.
- Enhances bio-safety with closed system.
- Handles large cell numbers (up to 10^{11}).
- Concentrates secreted proteins and antibodies up to 100X in the small volume of the extra-capillary space.
- Provides flexible control options with unique variable rate pump system.
- Ensures long life of the cartridge and facilitates nutrient and waste exchange across the fiber with positive-pressure displacement pumping system.

THE FIBERCELL® SYSTEM

The hollow fiber cartridge used in the FiberCell System has wavy fibers to eliminate dead space.

Enlarged cross section showing only three wavy fibers.



Cross section of cartridge showing the fiber ends potted in polyurethane so that medium flows only through the inside of the fiber.

THE FIBERCELL® SYSTEM: Hollow Fiber Cell Culture

A fundamentally different approach to cell culture is now available. Modeled after the mammalian circulatory system hollow fiber cell culture offers the most *in-vivo* like manner to grow cells in any laboratory.

Hollow fibers are small tube-like filters approximately 200 microns in diameter whose molecular weight cut-off can be between 5kd and .1µm. These fibers are sealed into a cartridge shell so that cell culture medium pumped through the end of the cartridge will flow through the inside of the fiber while the cells are grown on the outside of the fiber. These fibers then create a semi-permeable barrier of defined molecular weight cut-off (MWCO) between the compartment in which the cells are growing and the medium is flowing. Since the cells are attached to a porous support (the hollow fiber) rather than a non-porous plastic dish nutrients are delivered from the bottom layer of cells on upwards. Splitting of the cells is not required and cultures can be maintained for many months of continuous production. When the secreted protein is retained in the extra-capillary space it will accumulate to a concentration of up to 100 times higher than with conventional flask or roller bottle culture.

A single FiberCell® Systems hollow fiber cartridge is equivalent to harvesting 20-200 roller bottles daily.

Hollow fibers also provide a tremendous amount of surface area in a small volume. Cells grow on and around the fibers at densities of greater than 1 X 10^8 per ml. Hollow fiber cell culture is the only means to culture cells at *in vivo* like cell densities. Cell culture at high densities has the following benefits:

- Reduces serum requirements and facilitates adaptation to serum-free medium, including CDM-HD
- Increases the concentration of secreted product by 10 to 100 times
- Viral and parasitic infections proceed rapidly

FiberCell® Systems Hollow Fiber cell culture cartridges are the method of choice for the production of 10mg to gram quantities of recombinant proteins and monoclonal antibodies and the culture of 10^9 to 10^{11} cells.

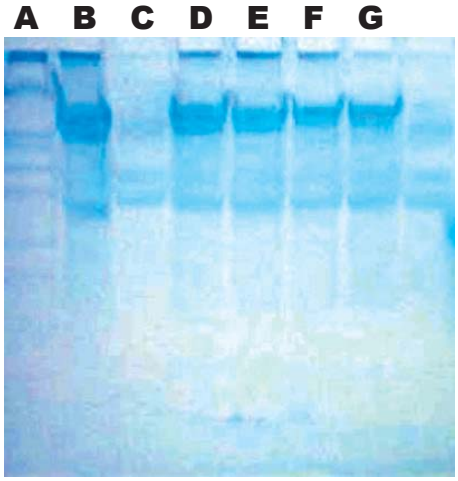
Protocols are available for monoclonal antibody production, recombinant protein production using CHO, 293 and other cell types, lymphocyte expansion, conditioned medium and endothelial cell culture under defined shear stress.

SUGGESTED APPLICATIONS

MONOCLONAL ANTIBODY PRODUCTION

The FiberCell® Systems hollow fiber cell culture system is the method of choice for the production of 100mg to 2 grams of antibody per month. The 20kd MWCO fiber allows the inhibitory factor TGF beta to diffuse away from the cells while retaining the monoclonal antibody in the small volume of the extra-capillary space.

- C2011 will produce between 10mg and 50mg of antibody every two days
- Low harvest volume of 15 to 70 mls
- Cartridges can be maintained for over 6 months of continuous production
- Endotoxin burden is 10X lower than ascites fluid, ideal for animal injections
- Easy adaptation to reduced serum, CDM-HD, or serum free medium
- Chimeric, humanized and non-murine antibodies can be easily produced

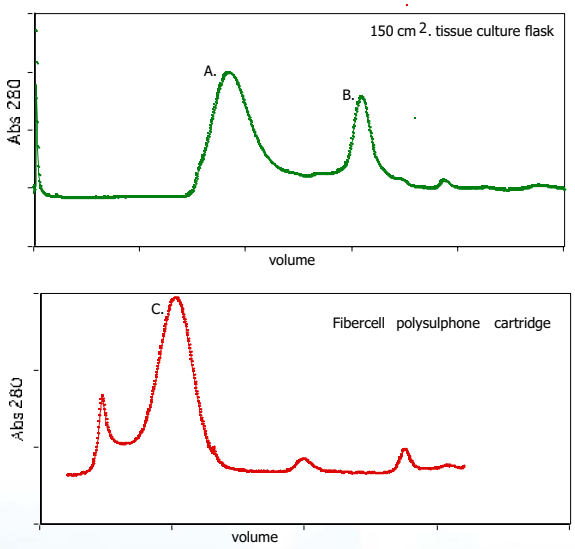


A) MW Standards
B, D, E, F, G) Harvested Antibody
C) Circulating serum free medium

SECRETED PROTEIN PRODUCTION

Current methods for the production of secreted biologicals such as recombinant proteins, cytokines and conditioned medium involve the use of inefficient *in-vitro* culture systems such as roller bottles or large numbers of tissue culture flasks. FiberCell® Systems hollow fiber culture systems facilitate the easy culture of large numbers of cells (1-5 X 10^{10}) replacing roller bottles or hundreds of flasks while concentrating the secreted protein 100X or more.

- Produce 1-10 mgs of recombinant protein per day
- Secreted proteins can be 100 times more concentrated vs. tissue culture supernatant
- Reduced serum requirements facilitates purification
- Small harvest volume for easy handling
- No splitting of cells required, cartridge maintenance just 15 minutes a day
- Cultures can be maintained for several months of production
- Optimal cell culture conditions can result in improved protein assembly and folding.



Comparison of a purified recombinant human hexeramized IgG produced from CHO cells grown in either a 150cm² tissue culture flask (upper panel) or a FiberCell polysulphone cartridge (lower panel). Gel filtration chromatography reveals incomplete polymerization of the hexeramized IgG produced in the flask. Both a highly polymerized hexeramized IgG (peak A.) and non-polymerized (peak B.) are observed. In contrast, the same protein produced in a FiberCell cartridge is expressed almost entirely in highly polymerized form (peak C.) 478 mg of purified protein were produced in 2 months in a volume of less than 5 liters using catalog #2018.

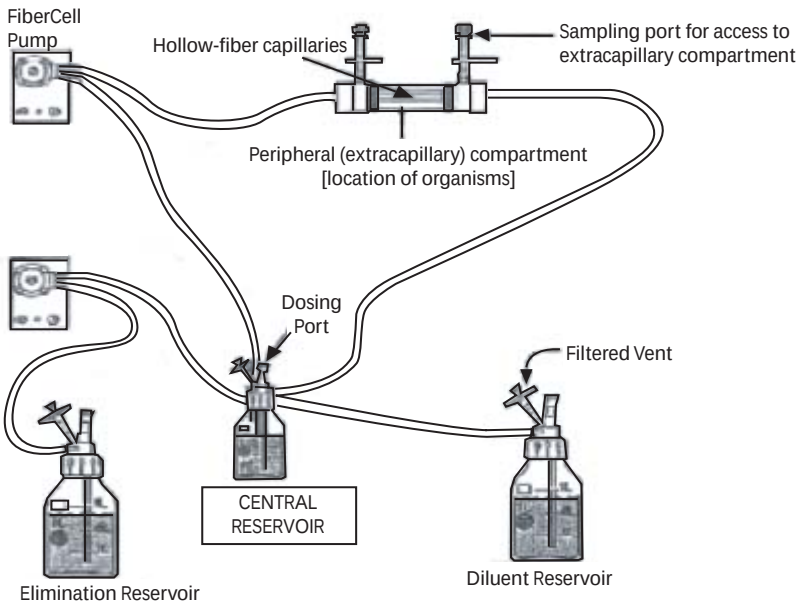
Data courtesy of Dr. Jim Arthos - Bethesda, MD.

LYMPHOCYTE CULTURE AND EXPANSION

FiberCell® Systems hollow fiber cell culture cartridges are ideal for the expansion of PBLs, lymphocyte subsets, HIV production and to perform large scale viral transductions of lymphocytes. The uniform distribution of the fiber bundle within the cartridge is optimum for suspension cell culture while the extremely high gross filtration rate of the hydrophilic polysulfone fiber insures adequate nutrient delivery and waste removal for rapidly growing cell types.

IN VITRO TOXICOLOGY

Hollow fiber cartridge systems from FiberCell Systems have been used to model pharmacologic bio-availability for different antimicrobial drugs and also to mimic dosage profiles that generate resistant organisms. Anti-cancer agents, anti-fungals, antibiotics, anti-virals (including anti-HIV) and anti-parasitic drugs have all been tested in the hollow fiber system. This system mimics *in vivo* drug dosage profiles in a rigorously controlled *in vitro* system composed of a hollow fiber cartridge and associated pumps and reservoirs. Bio-availability can be controlled in a precise fashion and any dosage profile can be evaluated.



ENDOTHELIAL CELL CULTURE

The FiberCell® Systems PS+ module (cat#4300-C2025) is especially designed for the culture and study of endothelial cells under flow. The PS+ fiber can easily be coated with extra-cellular matrix proteins (and/or cytokines and antibodies) to permit the attachment of endothelial cells to the interior wall of the fiber. The microprocessor controlled FiberCell® Systems Duet pump can be programmed to produce consistent and defined amounts of shear stress by regulating the flow of medium over the cells. This allows the study of endothelial cells in a more physiologic environment when compared to other methods. As a result, typical cell morphology is retained, a monolayer is formed and tight junctions are created. These culture conditions more closely mimic *in vivo* cell growth.

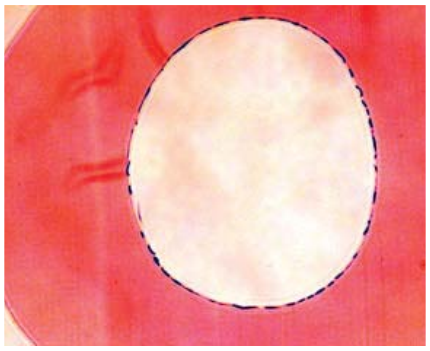


Figure 7 *Bovine Aortic endothelial cells grown on a matrix of fibronectin.*

The microporous nature of the fibers and the ability to control the extra-cellular matrix provides an ideal system for cellular co-cultivation with other cell types such as vascular smooth muscle or neuroglia.

- Inner surface area of the fibers is equal to a T75 flask
- Shear stress of .5 dynes/cm^2 to 25 dynes/cm^2 can be generated
- Approximately 100 micrograms of RNA can be isolated from each cartridge
- Most types of microscopy can be performed including immunohisto-chemical techniques

THE FIBERCELL® DUET™

FiberCell® Duet™ Specifications	
Dimensions	9.5 x 16.5 x 8" (w x l x h)
Weight	9.5 lbs
Voltage	100, 120, 220 (50 or 60 hz)
Flow Rate	1-200mls, continuously variable

The FiberCell® Duet™ The FiberCell Duet pump is engineered for flexibility and reliability. It is designed to support either one or two Fibercell Hollow Fiber Bioreactor cartridges and flow paths with up to two separate 2 liter medium reservoirs. The FiberCell Duet pump can generate a flow rate of 1 ml/min up to 200 mls/min for precise control of flow rate for endothelial cell culture applications and high maximum flow rates for most efficient production when using the larger C2003 and C2018 cartridges. It utilizes a new low voltage brushless motor for long term, low heat operation and operation with 100 volt, 120 volt and 220 volt electrical input. The Duet pump utilizes our proprietary positive-pressure displacement pumping systems that generates high flow rates without wear on the pump tubing permitting continuous operation of the cartridges for several months to one year ro more. The ergonomic, low-rise design provides areas to accommodate up to two 2 liter medium reservoir bottles.

- Occupies ½ shelf, 1/3 height of a standard CO^2 incubator
- Separate medium reservoirs eliminates cross contamination
- Low voltage power supply to minimize heat generation
- Versatile, can support small, medium and large cartridges (C2025, C2008, C2011, C2003, C2018, C5011)
- High flow rate for optimum culture using large cartridges
- Full Two year limited warranty

P3202	FiberCell Duet Pump, Universal Voltage, 100V, 120V, 220V

RESERVOIR CAP

The FiberCell® reservoir cap is a re-usable, autoclavable cap designed to fit onto a standard cell culture media bottle. The 33mm cap (cat# A1005) will fit onto standard glass medium bottle and the 38mm cap (cat# A1006) will fit onto standard plastic medium bottles.

A1005	33mm Reservoir Cap Assy
A1006	38mm Reservoir Cap Assy

CDM H-D

CDM-HD Serum Replacement is a chemically defined, protein free serum replacement that permits any basal medium such as DMEM to be used without serum. CDM-HD is designed specifically for the culture of cells at high density and optimized for use in our hollow fiber bioreactor systems. CDM-HD provides lot-to-lot consistency, simplifies purification and is an economical replacement for serum. It is available as a dry powder to make up one liter and is used at a concentration of 10%.

CDM-HD-1	Powder to make 1 Liter

FiberCell Systems offers additional technical support for our systems as well as for other hollow fiber bioreactor systems.

CARTRIDGE SPECIFICATIONS

Stock No.	Size	Surface Area	Fiber Type	Packing Density	ECS Volume	MWCO 50%	MWCO 95%	Maximum Cell#
C2025	Small	75cm^2	Activated PS	30%	1.5mL	0.1µm	0.1µm	10^8
C2008	Medium	2100cm^2	low flux PS	50%	15mL	5kd	20kd	10^9
C2011	Medium	2100cm^2	high flux PS	50%	15mL	20kd	100kd	10^9
C2003	Large	1.2m^2	low flux PS	50%	70mL	5kd	20kd	5×10^{10}
C2018	Large	1.2m^2	high flux PS	50%	70mL	20kd	100kd	5×10^{10}
C3008	Medium	2000cm^2	cellulosic	38%	12mL	10kd	5kd	N/A
C4005	X-Large	2.5m^2	low flux PS	50%	150mL	5kd	20kd	10^{11}
C4020	X-Large	2.5m^2	high flux PS	50%	150mL	20kd	100kd	10^{11}
C5011	Medium	2100cm^2	high flux PS	50%	15mL	20kd	100kd	2×10^9
Oxy 0.6	12mls	6000cm^2	Hydrophobic polyethylene	N/A	40mL	100kd	10kd	N/A

SUGGESTED APPLICATIONS FOR FIBERCELL® CARTRIDGES

C2025 0.1µm pore size for the highest exchange rates. Activated fiber for attachment of matrix proteins, cytokines and antibodies. Ideal fiber for endothelial cell and hepatocyte culture.

C2008 Low MWCO (5kd @ 50%) hydrophilic fiber for trapping smaller molecules. Suggested for recombinant proteins between 20kd and 100kd. Appropriate for suspension and adherent cell lines including CHO, HeLa and 293. Can support up to 10^9 cells and produce 1-2 mgs of recombinant protein per day.

C2011 High MWCO (20kd @ 50%) hydrophilic fiber for trapping larger molecules, hybridoma culture and lymphocyte culture. 20kd MWCO allows TGF beta and TNF alpha to diffuse away while retaining antibody. Can support up to 10^9 cells and produce 5-50 mg of monoclonal antibody every two days. Suggested for recombinant proteins larger than 100kd and will produce 1-2 mgs of protein per day.

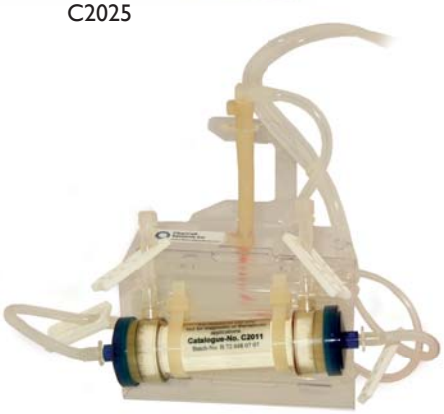
C2025

C2003 Low MWCO (5kd @ 50%) hydrophilic fiber for trapping smaller molecules. Suggested for recombinant proteins between 20kd - 100kd. Appropriate for suspension cell lines including CHO, HeLa and 293 cells. Can support up to 5×10^{10} cells and produce 5-10 mgs of recombinant protein per day.

C2018 High MWCO (20kd @50%, 95% @ 100kd) for trapping larger molecules in the range of 100kd and larger such as recombinant proteins from CHO, HEK 293 and other cell types. Can support up to 5×10^{10} cells and produce 5-10 mgs of protein per day.

C3008 utilizes a cellulosic fiber with a nominal MWCO of 5KD. C3008 is intended primarily for use for in vitro toxicology applications where organic based drugs may exhibit non-specific binding to other fiber types.

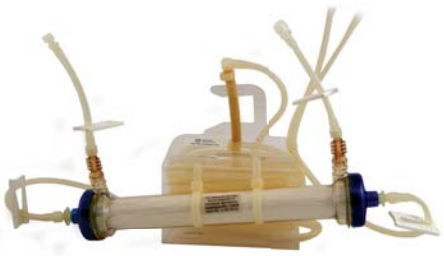
C4005 The C4005 cartridge is intended for use in larger hollow fiber cell culture systems from other manufacturers and does not include a flow path stand or oxygenator tubing. Side ports have 3" of tubing capped with luer fittings, end ports are 3/8" hose barbs. High gross filtration rate and polysulfone fiber are superior to cellulose acetate for recombinant protein production.



C2008
C2011

C4020 The C4020 cartridge is intended for use in larger hollow fiber cell culture systems from other manufacturers and does not include a flow path stand or oxygenator tubing. Side ports have 3" of tubing capped with luer fittings, end ports are 3/8" hose barbs. High gross filtration rate and polysulfone fiber are superior to cellulose acetate for recombinant protein and monoclonal antibody production.

C5011 Same sized cartridge as the C2011 but optimized for maximum monoclonal antibody production by increasing flow rate and doubling the amount of oxygenation tubing



C2003
C2018

Oxy 0.6 The Oxy 0.6 is a replacement oxygenator cartridge for 1.0 m2 oxygenators available from other manufacturers. It utilizes a new fiber with better gas transfer and reduced medium weepage. The fibers are also uniformly spaced apart using an inert matting system further improving gas transfer. Module comes with luer connections on both the end ports and side ports. Due to the geometry of the fiber medium is flowed through the ECS and gas is sparged through the inside of the fibers.

OUR MISSION

At FiberCell® Systems Inc. we are committed to providing easy-to-use hollow fiber cell culture systems that allow researchers and biotechnology companies to produce quantities of cells and cell products not possible using traditional cell culture methods. We are constantly creating new protocols and methods for the use of our hollow fiber bioreactors so that any laboratory can take advantage of the benefits offered by FiberCell® Systems Inc.

John J. S. Cadwell
President and CEO

To order contact:

FiberCell Systems Inc.
a better way to grow cells
905 W. 7th Street #334
Frederick, Md. 21701
+86-400-6762467 028-86653817

www.ahmedical.com; E-mail: ahmedical@verizon.net