美国健康医疗仪器国际公司

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

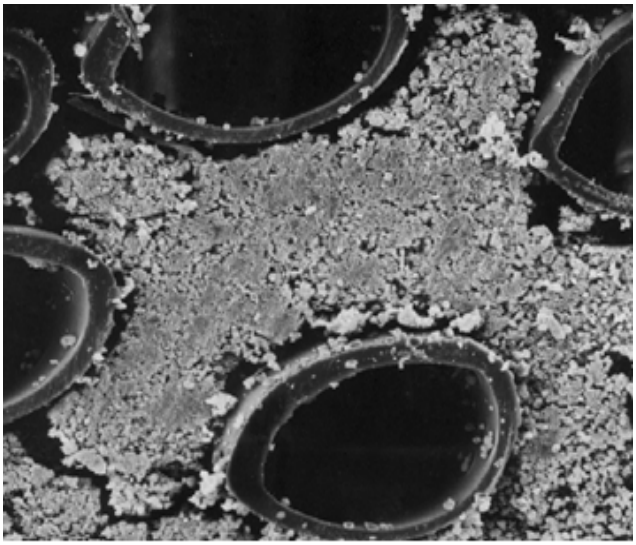
FiberCell Systems Inc. a better way to grow cells

小型、高效的 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

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;

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。

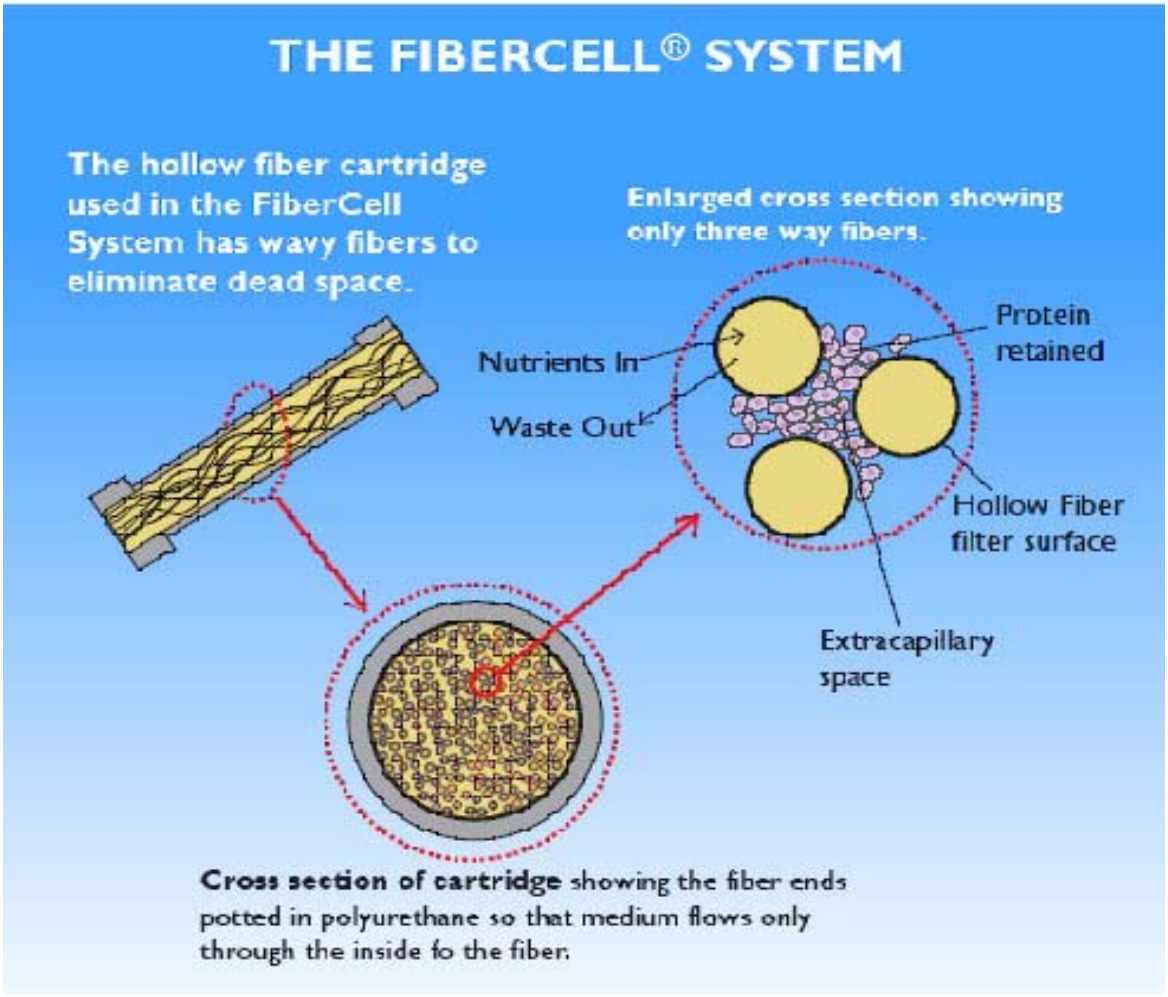


图 2 . **FiberCell 中空**纤维细**胞培养系**统 利用 hollow fiber membrane 隔离细胞与细胞培养液，在中空纤维的外壁（exra-capillary space, ECS）培养细胞的裝置。此裝置主要為模擬生物體循環系統中毛纤维的结构及功能；由具半透析性之多孔膜狀高分子-- polysulfone 天然亲水聚合物，拉成兩端有開口的纤维。將此種中空纤维裝入柱狀的塑胶容器中，其成品就像光纤排列在电缆中一样。

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

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

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。

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 100-500μg/mL；

- 体外毒性研究与分析 *In Vitro* Toxicology; 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)；

- 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；

- 病毒扩增：运用 Virus Production 可生产超过 1-3×10^{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；

- 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
  Stem cell and other cultures where extra-cellular matrix and cytokines can be important.

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

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。

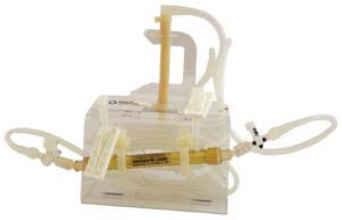
- 一天只需几分钟即可简易操作大量的细胞。
- 可进行为期六个月以上的长期细胞培养，而不需更换耗材
- 一组中空纤维细胞培养系统的产量相当于 20 瓶滚瓶产量
- 收取体积小，提高了抗体及蛋白质的浓度，方便纯化。
- 细胞密度高，可快速、一致的研究感染性病毒和其它微生物病原。

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

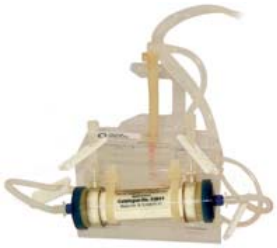
筒式培养系统规格：

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.

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.

X-LARGE Cartridges

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.

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

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.