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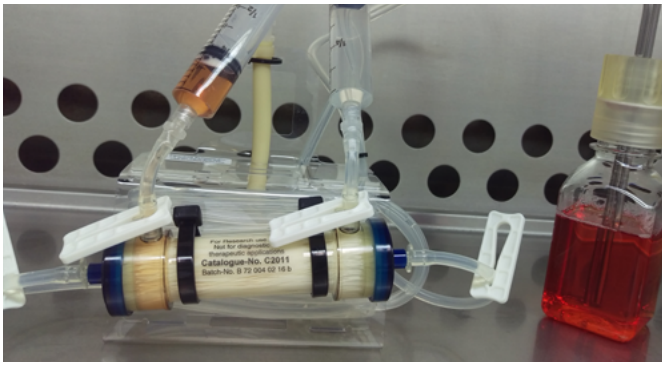
Fiber Cell Systems
A BETTER WAY TO GROW CELLS

THE ADVANTAGE OF HOLLOW FIBER BIOREACTORS



Three-Dimensional (3-D) Cell Culture

The *in vitro* culture of mammalian cells in the biomedical research laboratory is a near ubiquitous technique and a fundamental tool of biological research. It's a technique practiced by researchers of all levels, from high school students, university researchers on up to biopharmaceutical manufacturing scientists.

It is now appreciated that conventional cell culture techniques are not the most physiologically relevant way to grow cells or the optimal way to harvest biologics for production. With cells grown on nonporous plastic dishes, nutrients are delivered from the top down in flask culture, meaning that the bottom layers can become deprived. Cells need to be passaged every 2-5 days, which disrupts the cells and generates a lot of plastic waste.

Some cells can be cultured in suspension under constant stirring or agitation at low density, but again, under nonphysiologic conditions. The use of serum is not physiologically relevant, as the only time cells are exposed to serum rather than plasma is during wound states where the clotting factors have been activated. Lot-to-lot variations in serum composition and quality can also affect the relevance of cell culture results.

The Hollow Fiber Bioreactor

For the reasons outlined above, there is currently tremendous interest in 3-dimensional cell culture systems. The hollow fiber bioreactor (HFBR) is a high-density continuous-perfusion culture system consisting of a cartridge containing thousands of semi-permeable hollow fibers in a parallel array within a tubular housing fitted with inlet and outlet ports. The fiber bundles are sealed with a potting system at each end so that any liquid entering the ends of the cartridge will necessarily flow through the interior of the fibers. Cells are generally seeded outside of the hollow fibers in what is referred to as the extra capillary space (ECS), although cells may be seeded within the capillaries depending on the desired product/outcome. Culture media is circulated through the insides of the hollow fibers, allowing nutrients, gases, and waste products to diffuse both ways across the fiber walls. After passing through the cartridge, the culture medium is oxygenated and re-circulated to the cartridge. HFBRs offer a unique environment for a more *in vivo*-like cell cultivation and cell co-cultivation. They present a 3-D environment similar to the conditions found in the body, and they support the continuous control of oxygenation levels, medium composition, and drug concentration.

There are three fundamental characteristics that differentiate hollow fiber cell culture from any other cell culture method:

- Cells are bound to a porous support, much as they are *in vivo*
- The molecular weight cut off (MWCO) of the hollow fiber filter can be controlled (ranging from 5 kD to 0.1 μM)
- A very high surface area-to-volume ratio (>150 cm^2 per mL)

Cells Bound to a Porous Support

Cells bound to a porous support do not require splitting or passaging, and HFBR cultures can maintain viability and productivity in a postconfluent manner for extended periods of time. Furthermore, cells in an HFBR are not subject to shear, and necrotic cells do not become apoptotic and do not release significant cytoplasmic proteins or DNA into the harvested product. This provides a cleaner culture environment, more accurate culture parameter monitoring, and a harvest that is simplified and easier to handle for downstream assays and purification.

Controlled Molecular Weight Cut-Off

By altering the MWCO, secreted products can be retained within the ECS to concentrations up to 100 times higher than in standard cultures. The effects of cytokines on the cells can also be controlled. Secreted recombinant proteins can be selectively retained and concentrated while cytokines and other factors that facilitate cell-to-cell interactions can be concentrated, as well. Small molecule drugs can easily exchange across the fiber and rapidly reach equilibrium while larger bacteria and cells are retained.

High Surface-to-Volume Ratio/High Cell Densities

The small diameter of the fibers (200 μM) generates an extremely high surface-area-to-volume ratio of 150-200 cm^2/mL within the cartridge. The exchange of nutrients and waste products is high enough to support cell densities of 1-2 x 10^8/mL, approaching *in vivo* tissue-like densities. This high cell density allows for the simplification of the cell culture medium, with differences in medium formulation performance mitigated by the cells' own secreted factors. In fact, the more complex and sometimes more expensive a cell culture medium is, the less effective it is in a hollow fiber cell culture environment.

Protein-Free, Chemically Defined Culture Medium

Animal serum has inherent problems including risk of contamination and variability in both production and cell-based assay performance. There are a number of nonculture-related limitations as well including high and variable costs and interference of high protein concentrations with culture analysis. HFBR culture conditions are tailored for use with FiberCell Systems' CDM-HD, a protein-free commercially available serum replacement which requires high cell densities. CDM-HD usage results in cleaner product harvests, simpler purification processes, and better-defined culture environments.

In vitro cell culture aims to recapitulate the *in vivo* environment as closely as possible, as culture conditions have been shown to profoundly affect the quantity and quality of secreted products from mammalian cells. Biologically relevant models of *in vivo* processes are also dependent upon mimicking the *in vivo* environment as closely as possible. The 3-D and more *in vivo*-like cell culture conditions present in a HFBR have been clearly demonstrated.

Take Advantage of our Advances

Hollow Fiber Bioreactors: A Better Way to Grow Cells

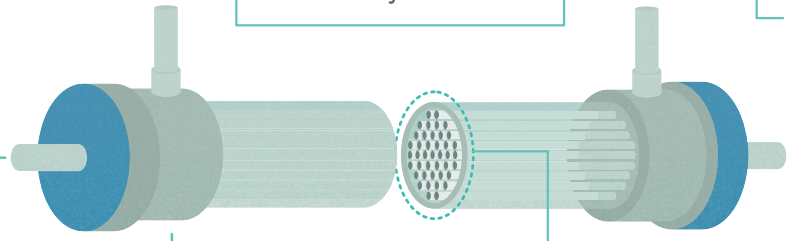
A closer look at the FiberCell Systems cartridge

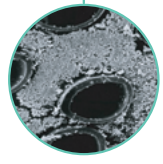
Cells seeded in the extra-capillary space grow post-confluently for many months

FiberCell Systems' chemically defined CDM-HD is simplified and optimized specifically for high density culture

Ideally suited for continuous collection of secreted products at high concentrations

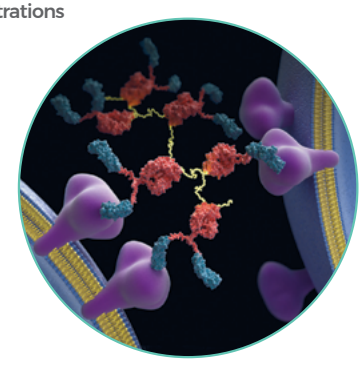
Hollow fiber bioreactors (HFBR) continuously recirculate medium to provide nutrients and oxygen while removing waste and CO_2





The closed, bio-safe system is cGMP compliant

The unique chemical environment around the cells is controlled by the MWCO of the fibers



Ideal for producing difficult-to-express proteins

APPLICATIONS

3-D Cell Culture

The medium-sized cartridge offers 3,000 cm^2 of surface area, equivalent to 40 T-75 flasks, but can support up to $2x10^9$ cells

Monoclonal Antibody Production

In the HFBR, hybridomas can yield up to 100 mg of antibody every two days, for months of culture

Recombinant Protein Production

Typical protein harvests from FiberCell Systems' cartridges contain 100 µg/mL/day or higher. Daily harvests are 2 mg to 10 mg a day.

Exosome Production

Gram quantities of exosomes can be produced in HFBRs

Cell Co-culture

The PVDF fiber allows various matrix proteins to be bound to its surface

Learn more at **www.fibercellsystems.com**

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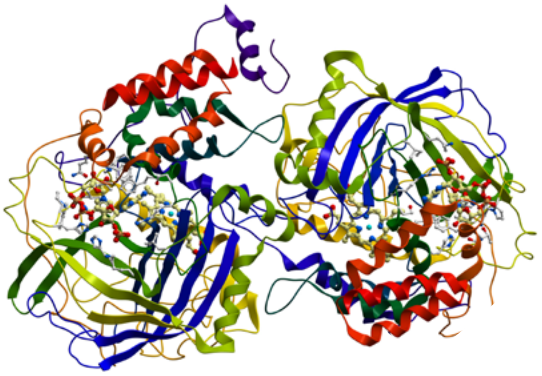
Expression of Recombinant or Difficult-to-Express Proteins in Mammalian Cells



Cultured mammalian cells have become the predominant platform for the production of recombinant proteins for clinical applications due to their capacity for proper protein folding, assembly and post-translational modification. Protein quality is also important in the research laboratory for proper biological activity and for potentially therapeutic and difficult-to-express proteins. However, the production of recombinant proteins and conditioned medium from mammalian cells in the typical research laboratory can be such a cumbersome process that many avoid using them as expression systems. Furthermore, while well-understood, robust, and convenient, classical batch-style 2-D cultures are not very biologically relevant systems. Hollow fiber bioreactors (HFBR) provide a more physiologic, *in vivo*-like 3-D environment than other cell culture methods, and they can also result in improved protein folding and more uniform post-translation modifications over time.

Advantages of Protein Expression in Mammalian Cells

Expression in mammalian cells remains the primary methodology for therapeutic protein biopharmaceutical production of approved products, with hundreds more in corporate pipelines. Most of these proteins are expressed in Chinese Hamster Ovary (CHO) cells, but other cell lines such as mouse myeloma (NSO), baby hamster kidney (BHK), and human embryonic kidney (HEK-293) cells are commonly used as well.

The production of recombinant proteins at the manufacturing scale has been refined over the years to follow a standard scheme, in part to facilitate regulatory compliance. The producing cells are adapted to suspension culture (or attached to microcarriers) to allow for large scale suspension culture in stainless steel stirred-tank vessels up to 10,000 or 20,000 L in size. Cell culture medium formulations are iteratively tested and optimized along with control parameters such as pH, dissolved oxygen, and CO_2. Cells are seeded into the reactor where they consume the medium. When the medium is exhausted, the secreted product is harvested from the reactor. Production of mammalian-expressed proteins in a hollow fiber bioreactor does not require adaptation to suspension culture, and medium optimization is not required in the initial stages, saving valuable time.

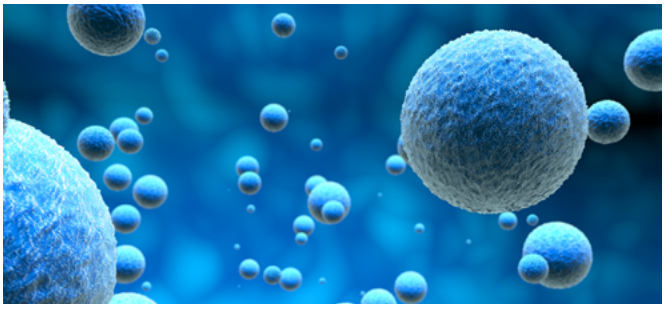
Hollow Fiber and Difficult-to-Express Proteins

Protein research at the laboratory scale is the basis for the development of therapeutic products. It is critical that these be produced in a form that retains all of the characteristics of the final product so that results seen in the research lab can be extended to the clinic. Expression of difficult-to-express proteins in mammalian systems is efficient and cost effective in a hollow fiber bioreactor. There are many advantages to working with a protein that is correctly folded with tertiary structure intact, including the maintenance of proper solubility, bioactivity, glycosylation (thus preventing potential antigenicity and immunogenic reactions), and pharmacokinetics.

Difficult-to-express proteins can be proteins that are expressed at low titers by mammalian expression systems. They are also defined as highly complex proteins: highly glycosylated, with high levels of posttranslational modifications and, in some cases, consisting of several subunits. Case studies using HFBRs have demonstrated superior expression of these types of proteins such as the hexamerized IgG and the IL-15 ligand-receptor complex. The next generation of therapeutic proteins include bi-specific and tri-specific antibodies, protein structures that are the creation of bio-engineers and not found in the templates of nature. These proteins require the *in vivo*-like cell culture conditions and concentration that HFBRs and mammalian expression systems provide. A hollow fiber bioreactor system can allow any laboratory totake advantage of the superior folding, glycosylation, and complete post-translational modifications that only expression in mammalian cells can provide.

In vivo-like cell densities, constant provision of nutrients and removal of waste products result in complete and uniform post-translation modifications over long term cultures. A hollow fiber bioreactor is therefore ideal method for the production of recombinant proteins from mammalian cells, and is particularly useful for the production of difficult-to-express proteins.

Exosome Production



Exosomes are small lipid-membrane vesicles (80-120 nm) of endocytic origin generated by fusion of cytoplasmic endosomal multi-vesicular bodies with the cell surface. Protein and miRNA is exported outside cells via exosomes into the circulatory system. Exosomes may serve to shield miRNA contained within them from degradation, allowing them to serve as intercellular communication vectors.

Until recently, exosomes were simply considered to contain intracellular garbage. However, exosomes have garnered tremendous interest over the past few years, showing promise for cancer therapy and disease biomarker research. Increasing evidence suggests that tumor cells release increased amounts of exosomes, which may influence tumor initiation, growth, progression, metastasis, and drug resistance. In addition, exosomes transfer messages from tumor cells to immune cells and stromal cells, contributing to the escape from immune surveillance and the formation of the tumor microenvironment. Their complexity permits them to be a proxy of cell health in culture. Finally, exosomes have received significant attention in regenerative medicine, as stem cell-secreted exosomes secreted have demonstrated wound healing and regenerative effects.

Exosomes have very high specific activity, but progress in understanding and applying them has been impeded by their low availability and difficulty in producing large quantities for experimentation. In laboratory-scale production, any number of culture, harvest and purification approaches have been used. The most common batch-mode protocol can entail a final stage of hundreds of large flasks and many liters of medium to process. Current isolation protocols utilize ultracentrifugation, which is not practicable for large-scale production. The composition and activity of exosomes reflect the physiologic state of the cells when secreted ,and this method is far from physiologic or relevant to *in vivo* conditions, as it can take several rounds of splitting and culture expansion to attain this final production cell-mass. This method is wasteful, time consuming, space consuming, and not amenable to scale up for clinical applications. These methods can pose technical challenges, as cells undergoing apoptosis just prior to harvest, contaminating the batch with difficult-to-remove membrane fragments and protein aggregates.

Hollow Fiber Bioreactors for Exosome Production

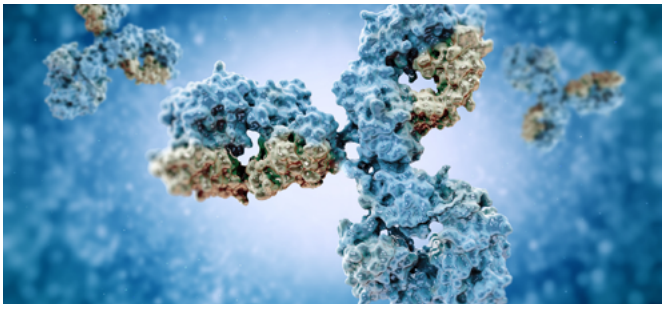
HBFRs offer significant advantages over flask culture, especially for the collection and concentration exosomes under physiologic cell-culture conditions. HFBRs are the most efficient way to culture large numbers of cells as they grow. They provide a high amount of surface area so large numbers of cells can be supported at high densities. The molecular weight cut off of the fiber allows nutrients and waste products to pass through the fibers while retaining larger secreted products and exosomes in the small volume of the extra-capillary space (ECS), where they accumulate and concentrate. If serum is required for production it can be used in the circulating medium only, while the ECS containing the cells and secreted exosomes can be maintained serum free. Endogenous exosomes in serum cannot cross the fiber, but the factors in serum that support cell growth can. The HFBR can facilitate the use of a protein-free medium such as CDM-HD. The cells are also bound to a porous support so that splitting is not required; cells are free to grow post-confluent and collection of secreted products can be maintained over several weeks or months of continuous production.

Case Study: HEK-293 Exosome Production

HEK-293 culture expressing heterodimeric IL-15 was maintained in a hollow fiber bioreactor for over 4 months of continuous production, with 3 harvests of 20 mL per week. The HEK-293 bioreactor culture yielded an equivalent number of exosomes per harvest as seventy T225 flasks. CD63 and Alix were greatly enriched from the bioreactor compared to flask culture. EV/protein ratio was 10-fold higher in harvests from the bioreactor suggesting a significant reduction in contaminating cell membrane fragments. Purified HEK-293 cells retained their IL-15 biological activity.

Hollow fiber bioreactors from FiberCell Systems represent a more *in vivo*-like way to produce exosomes of both the quality and quantity required for laboratory research as well as clinical relevance in a closed, single-use system. Exosomes can be highly concentrated and experience reduced contamination from intracellular proteins and membrane fragments as a result of reduced apoptosis. FiberCell HFBRs have the capability of producing gram quantities of exosomes using currently available cartridges. A hollow fiber bioreactor is the ideal method for the production of exosomes without serum and under cGMP-compliant conditions, and represent a paradigm shift in advancing both exosome research and clinical applications.

Monoclonal Antibody Production from Hybridomas



César Milstein was awarded the 1984 Nobel Prize, jointly with his former postdoctoral fellow Georges J. F. Köhler, for developing the hybridoma method of producing monoclonal antibodies. Today, monoclonal antibodies continue to be at the cutting edge of medical research as scientists continue to discover uses for them in both diagnostic and therapeutic applications. Nowhere has the impact been greater than in the field of oncology, where researchers are developing monoclonal antibodies that bind specifically to tumors and target oncolytic drug delivery.

Hybridoma Cells Inhibit Their Own Proliferation

Milstein recognized that when you fuse a B cell with a cancer cell to generate a hybridoma, you create a cell that inhibits its own growth. One of the ways that cancer cells locally immune suppress is by secreting a factor called TGFβ, which inhibits the growth and activity of B cells. By removing TGFβ while retaining IgG, one can greatly enhance the production of monoclonal antibodies in cell culture. Based upon this principle, HFBRs are the ideal system for the production of monoclonal antibodies from hybridoma cell lines as they allow TGFβ to diffuse away from the cells, while concentrating the antibody to high levels inside the hollow fiber bioreactor itself.

Recapitulating *In Vivo* Conditions for High Fidelity Protein Production *In Vitro*

The production of secreted products (e.g., recombinant proteins and monoclonal antibodies) from mammalian cells is generally performed in the laboratory by standard flask, roller, or spinner culture. Only recently has it been recognized how culture conditions can dramatically impact protein quality. In conventional culture systems, cells are adapted to nonphysiologic low-density plastic-bound 2-D or suspension-culture conditions. This adaptation can affect the quality and purity of the antibody or protein produced.

The HFBR is a high-density, continuous perfusion culture system that can maintain post-confluent cell viability for extended periods of time – months or longer. For example, one hybridoma was reported to maintain productivity for over one year of continuous culture. The lack of passaging and the maintenance of biologically homeostatic culture conditions results in improved folding and complete and uniform post-translational modifications. Furthermore, the more *in vivo*-like growth conditions and lack of shear within a HFBR result in significantly reduced apoptosis. Rather, the majority of cells become necrotic and do not release cytoplasmic proteins, lysozymes, or DNA into the culture medium, resulting in a product that is cleaner and easier to purify from the bulk harvest. Finally, the ability to control fiber molecular weight cutoff allows for the retention of higher concentrations of the desired products, controls cytokine effects, and facilitates the selective removal of inhibitory TGFβ.

Concentrated Product for High Yields and Easier Purifications

HFBR cell culture results in protein and antibody concentrations that can be 100x higher than those found in flask or spinner culture, with almost no contaminating proteins. HFBR culture conditions result in improved protein folding and more uniform and complete glycosylation patterns over time. Finally, the higher cell densities supported by HFBRs allow lower serum concentrations or a simplified, protein-free serum replacement such as CDM-HD, resulting in much cleaner harvests and simplified purification. Since no animal host is required (such as mice or nude mice), chimeric (mouse/human) and non-murine antibodies such as rabbit, rat and hamster can be easily produced.

HFBRs are an effective method for producing milligram to gram quantities of monoclonal antibodies and recombinant proteins. The harvested product is concentrated and free of contaminating proteins, DNA, RNA, and proteases. Cultures can be maintained for long periods of time and the scalability of the system is determined by length of culture, not new equipment. The HFBR can give the power of hollow fiber cell culture to any laboratory.