Hollow Fiber Bioreactors and Difficult-to-Express Recombinant Proteins

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Difficult-to-express proteins are generally considered to be recombinant proteins that are expressed at low titers in mammalian systems, but in fact also include highly complex, highly glycosylated, large and unstable proteins as well. The solubility, immunogenicity, and bioactivity of a protein can be superior when expressed in mammalian cells versus other hosts such as bacteria, insect cells and yeast. Cultured mammalian cells are the preferred platform for the production of recombinant proteins for clinical applications due to their capacity for proper protein folding, assembly and post-translational modifications. This superiority of protein quality is also important in the research laboratory to ensure proper biological activity and especially for potentially therapeutic proteins.

The expression of recombinant proteins in mammalian cells in the typical research laboratory can be such a cumbersome process that the use of mammalian expression systems is avoided. Large numbers of plates, flasks or roller bottles are required, and large volume spinner flasks or the use of an expensive stirred tank bioreactor may be needed for scale-up. Low density suspension cultures or 2-D flask based processes, and the use of serum is inherently non-physiologic. While well-understood, robust, and convenient, classical batch-style 2-D cultures are not biologically relevant systems. Proteins are expressed at low concentrations, post-translational modifications may be incomplete, and the presence of serum or other proteins in the medium complicates purification.

Hollow fiber bioreactors (HFBR) provide a more physiologic, in vivo-like 3-D environment than other cell culture methods, and can also result in improved protein folding and more uniform post-translation modifications over time in a continuous, perfusion based process.

Advantages of Hollow Fiber Bioreactors

- No splitting of cells is required, maintenance is just 15 minutes a day
- Cultures can be maintained for several months of continuous production
- Optimal cell culture conditions can result in improved protein assembly and folding
Small harvest volume for easy handling and more efficient down-stream processing
- Relatively small number of cells are required for seeding, no seed reactor is required
- Reduced cell lysis, less host cell protein and DNA contamination
- Constant replenishment of nutrients and removal of waste products, such as ammonia, maintains homeostatic cell culture conditions. Removal of ammonia is especially important for proper glycosylation.
- Single use, cost effective, and produces less plastic waste
- Eliminates requirement for adapting cells to suspension culture or medium optimization
- Use of protein-free CDM-HD simplifies purification

Three fundamental characteristics differentiate hollow-fiber cell culture from any other method:

1) Cells are perfused and bound to a porous matrix much as they are in vivo—not a plastic dish, micro carrier bead, or other impermeable support.
2) The molecular weight cut-off (MWCO) of the fiber can be controlled.
3) There is an extremely high surface area-to-volume, 150 cm² to 200 cm² per mL, resulting in high cell densities.

Figure above: In vivo-like environment of the hollow fibers

CELLS ARE BOUND TO A POROUS SUPPORT MUCH AS THEY ARE IN VIVO

Cells bound to a perfused porous support do not require splitting and passage number becomes irrelevant. Cells in a HFBR maintain viability in a post-confluent manner for extended periods of time—months or longer. The lack of passaging and the maintenance of biologically homeostatic culture conditions result in improved folding
and complete and uniform post-translational modifications. The more in vivo-like growth conditions and lack of shear within a HFBR also result in significantly reduced cell lysis. Host cell proteins, lysozyme and DNA are not released into the culture medium resulting in a product that is cleaner and easier to purify.

THE MOLECULAR WEIGHT CUT-OFF OF THE FIBER CAN BE CONTROLLED

HFBRs are available with a 5 kD and 20 kD MWCO. Secreted proteins are retained and concentrated within the ECS (extra-capillary space) of the cartridge 50 – 100 X higher concentrations than flasks, and the effects of cytokines can also be controlled.

THERE IS AN EXTREMELY HIGH SURFACE AREA-TO-VOLUME RATIO

The small diameter of the fibers (200 µm O.D.) creates a surface-area-to-volume ratio of 150 - 200 cm²/mL volume. When coupled with the high gross filtration rate of the polysulfone fibers, the exchange of nutrients and waste products across the fibers is very rapid. Cell densities of 1 - 2 X 10⁸ cells per mL are achieved; close to in vivo-like densities.

CDM-HD SUPPORTS MAMMALIAN CELLS UNDER HIGH DENSITY HOLLOW FIBER CELL CULTURE CONDITIONS

CDM-HD is a chemically defined, protein-free, animal component free, cGMP compliant serum replacement optimized for the HFBR. The specific high-density cell culture conditions inside a HFBR are different enough from standard culture conditions that a cell culture medium can be simplified and optimized to take advantage of these conditions. CDM-HD can support cell cultures in a HFBR but not in flasks or other low density culture systems. It is a direct manifestation of the different cell culture conditions found in an HFBR.

The use of CDM-HD in a HFBR eliminates contaminants found in serum such as lipids, endotoxin, proteins, viruses and other adventitious agents. The use of protein-free media results in much cleaner harvests of products and simplified purification. Yields can be improved by reducing the number of purification steps required. Chemically defined CDM-HD also simplifies regulatory compliance.

A HFBR from FiberCell Systems is the ideal method for the in vitro production of 25 mg on up to gram quantities of a recombinant protein.
CASE STUDIES

Production of a Hexeramized Recombinant IgG from CHO Cells in the FiberCell Systems C2018 Cartridge

Production of a hexeramized IgG consisting of 6 IgG1 subunits held together by 3 IgA tails was expressed in a CHO cell line and produced in a 1.2 m², 20 kd MWCO hollow fiber bioreactor. This is a classic example of a difficult-to-express protein; a very large, highly glycosylated, complex, engineered structure not seen in nature. When grown in flask culture (top trace) about 40% of the protein is expressed as an incompletely folded monomeric sub-unit. When the same cells are culture in the FiberCell Systems HFBR module, more than 90% of the protein is expressed as a properly folded hexamer. This is due to the superior cell culture conditions inside the hollow fiber cartridge. 478 mg of purified protein was produced in 8 weeks with a harvested volume of less than 5 liters.

Data above, courtesy of Dr. James Arthos, NIH, Bethesda, Md.

Production of IL15 Receptor Complex

Interleukin 15 receptor complex is one of the ultimate difficult-to-express proteins. It consists of two subunits, held together by hydrophobic interaction and is 45% glycosylated. The IL15-RC heterodimer demonstrates superior pharmacokinetics and in vivo bioactivity compared to the single chain IL15 expressed in e. coli. Expression in standard cell culture systems is problematic. Efficient production of this non-

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covalently linked but stable heterodimer in HEK293 cells is demonstrated in a 5 kDa MWCO HFBR. Cell supernatants (20 mL) were harvested daily for up to 5 months and assayed for IL levels by ELISA.

This is an important cytokine with potential clinical applications as a lymphocyte growth and activation factor. Although monomeric E. coli-produced IL is in the initial stages of clinical testing, this form of the molecule poses multiple challenges for clinical use due to its instability and rapid plasma clearance. HEK293 cells produce correctly folded and glycosylated human IL-RC heterodimeric cytokine that shows greater stability and longer half-life. The superior bioactivity of IL15-RC in the heterodimeric form is the result of the presence of the IL-receptor contributing to increased stability of the protein in vivo. These properties offer the potential to allow lower and less frequent dosing and simpler delivery methods, with increased convenience for both patients and caregivers.

Data above, demonstrating consistent, continuous product of the IL over 150 days of culture, and no change in glycosylation or assembly over this time. (2)

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 proteins and especially difficult-to-express proteins in mammalian systems is efficient and cost effective in a HFBR. There are many advantages to working with a protein that is correctly folded with tertiary structure intact.

1) Solubility is maintained
2) Proper bioactivity
3) Antigenicity and immunogenicity characteristics retained
4) Improved half-life and pharmacokinetics

The new generation of biotherapeutics under development are increasing in size and complexity, with higher specific activities. As new therapeutic strategies are developed, bioengineering moves beyond copying that which is expressed naturally and begins to use novel and unique structures not found in nature, but created in the mind of the bioengineer. (3) This new class of proteins includes bi-specific T-cell engager (BITE) antibodies and tri-specific killer engagers (TRIKE) which utilize unique, newly created structures for their novel functionality.

A HFBR system from FiberCell Systems allows any laboratory to take 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, and total lack of shear result in complete and uniform post-translation modifications over continuous production cultures. HFBRs are an effective method for producing milligram to gram quantities of recombinant proteins. The harvested product is concentrated and contaminating proteins, DNA, RNA, and proteases are reduced significantly. The use of CDM-HD renders the medium economical and chemically defined. Cultures can be maintained for long periods of time, meaning that scalability of the system is determined by length of culture, not new equipment. Protein-free medium and high concentration product simplifies downstream processing, and can result in increased yields by reducing the steps required for purification. A HFBR from FiberCell Systems is the ideal method for the production of 50 mg on up to gram quantities of recombinant proteins from mammalian cells, and is particularly useful for the production of difficult-to-express proteins.
REFERENCES


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