

# Production of Recombinant Proteins and Monoclonal Antibodies in Hollow-Fiber Bioreactors

The production of secreted products from mammalian cells such as recombinant proteins and monoclonal antibodies is generally performed in the laboratory by standard flask, roller, or spinner culture. Only recently have we begun to understand how culture conditions can dramatically affect protein quality. In traditional culture systems, cells that originally grew attached to a porous matrix at high densities with little variability in per-cell nutrient and oxygen supply were adapted to low-density styrene-bound or amorphous suspension culture. While well-understood, robust, and convenient, classical batch-style 2-D cultures on nonporous supports or suspension culture in other devices are really not very biologically relevant models. Cell culture conditions can affect the quality and purity of the antibody or protein produced.

In order to more closely approximate *in vivo* cell growth conditions, Richard Knazek developed the hollow-fiber bioreactor (HFBR) in 1972.<sup>1</sup> The HFBR is a high-density, continuous perfusion culture system (see *Figure 1*). It consists of thousands of semi-permeable hollow fibers in a parallel array within a tubular housing or cartridge fitted with inlet and outlet ports. These fiber bundles are potted 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 within the cartridge, but outside of the hollow fibers in what is referred to as the extra capillary space (ECS).

Culture medium is pumped inside the hollow fibers, allowing nutrients and

waste products to diffuse both ways across the fiber walls (see *Figure 2*). Once it has passed through the cartridge, the culture medium is oxygenated and recirculated to the cartridge. Three fundamental characteristics differentiate hollow-fiber cell culture from any other method: 1) Cells are bound to a porous matrix much as they are *in vivo*—not a plastic dish, microcarrier, or other impermeable support; 2) the molecular weight cut-off (MWCO) of the support matrix can be controlled; and 3) there is an extremely high surface area-to-volume ratio (150 cm<sup>2</sup>/mL or more).

1. Cells are bound to a porous support much as they are *in vivo*. It is not necessary to split cells. Cells in this perfusion system maintain viability and production-relevant metabolism in a postconfluent manner for extended periods of time—months or longer. For example, one hybridoma was reported to maintain efficacious productivity for over one year of culture. The more *in vivo*-like growth conditions afforded by HFBRs result in significantly reduced apoptosis.<sup>2</sup> The majority of cells that become necrotic will not release cytoplasmic proteins or DNA into the culture medium, again resulting in a product that is cleaner and easier to purify from the bulk harvest.
2. The molecular weight cut-off of the fiber can be controlled. Desired products can be retained to significantly higher concentrations, and the effects of cytokines can also



Figure 1 Hollow-fiber bioreactor.

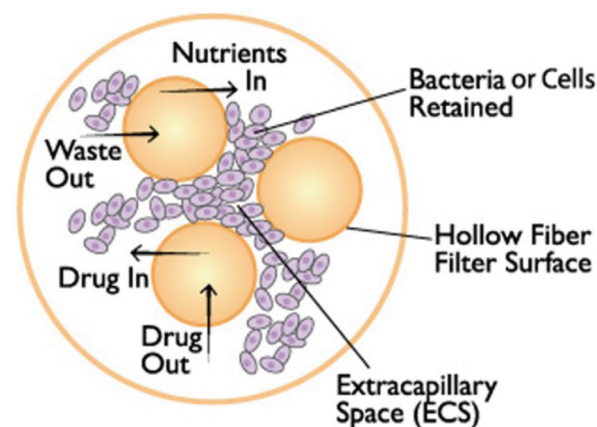


Figure 2 Hollow-fiber cell culture process.

be controlled. This is especially important for hybridoma culture, in which the inhibitory cytokine transforming growth factor (TGF) beta can be selectively removed from the culture while the secreted antibody is retained.

3. There is an extremely high surface area-to-volume ratio. The small diameter of the fibers (200  $\mu\text{m}$ ) generates a surface area-to-volume ratio in the range of 100–200 cm<sup>2</sup>/mL. When coupled with the high gross filtration rate of **FiberCell® Systems** (Frederick, MD) polysulfone fibers, the exchange of nutrients and waste products is very rapid. Cell densities of 1–2  $\times 10^8$  or more are achieved, close to *in vivo* tissue-like densities. A 20-mL cartridge will support as many cells as a 2-L spinner flask or 20–40 roller bottles. High cell densities produce more protein per milliliter volume, and facilitate adaptation to lower serum concentrations or a simplified, protein-free serum replacement such

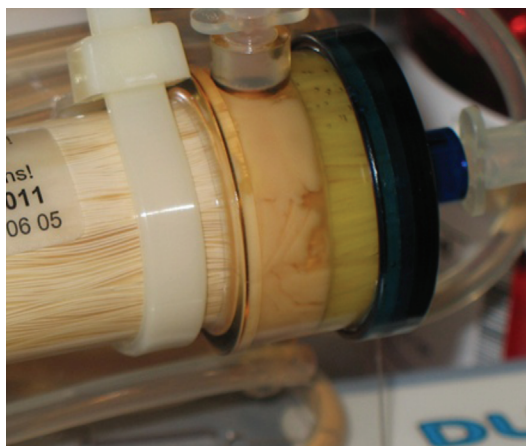


Figure 3 Cells growing at high density in a **FiberCell Systems** hollow fiber bioreactor cartridge.

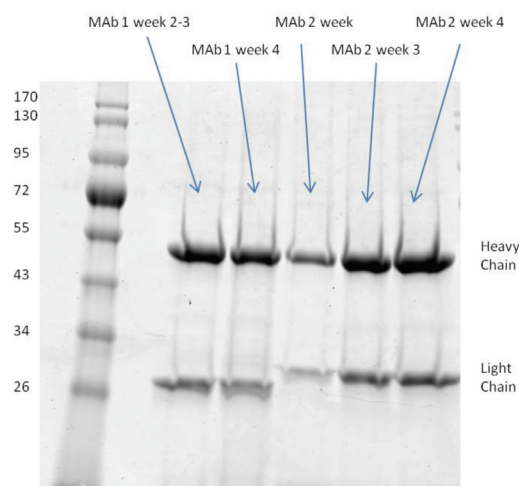


Figure 4 Monoclonal antibodies produced from a hybridoma cell line using **FiberCell Systems** CDM-HD. MAb 1—2.6 mg/mL week 2 and 3 harvest: (40 mL); MAb 1—3.2 mg/mL week 4 harvest: (20 mL); MAb 2—0.8 mg/mL week 2 harvest: (25 mL); MAb 2—3.2 mg/mL week 3 harvest: (20 mL); MAb 2—3.0 mg/mL week 4 harvest: (25 mL). (Data courtesy of Dr. Erin Bromage, University of Massachusetts, Amherst.)

as CDM-HD (**FiberCell Systems**). The use of protein-free media results in much cleaner harvests of products and simplified purification.

CDM-HD is a protein-free, animal-component-free, chemically defined serum replacement that is optimized for cell culture at high densities, such as is found in hollow-fiber bioreactors (see Figure 3). It contains specific micronutrients, amino acids, free iron, additional buffering capacity, and no surfactants as part of its proprietary formulation. It is supplied as a dry powder and is intended to be

used as a serum replacement for most cell types.

The above features of hollow-fiber cell culture result in protein and antibody concentrations that can be 100× higher than those found in flask or spinner culture, with almost no contaminating proteins from either the cell culture medium or the cells themselves. The more in vivo-like cell culture conditions can also result in improved protein folding and more uniform glycosylation patterns over time. Since it is a continuous perfusion system, the amount of protein produced is determined both by the length of time the culture is maintained and by such parameters as the clone's specific productivity or size of the cartridge.

### Case study 1: Mouse monoclonal antibody (MAb)

**FiberCell Systems** HFBR allows the production of about 50–200 mg of antibody per week using the medium-sized reactor (cat. no. C2011 or C5011). Two MAbs produced in Dulbecco's modified Eagle's medium (DMEM) and CDM-HD media were treated as above. The protein concentration of the dialyzed supernatants was quantified and their purity was checked on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (see Figure 4). (Note: The gel in Figure 4 represents the actual supernatant harvest that was simply dialyzed. No purification was performed; the antibody was harvested in a very clean state with little or no contaminating protein.)

### Case study 2: Rabbit MAbs

Rabbit monoclonal antibodies can be especially difficult to produce. Secretion levels can be so low as to be nearly undetectable in flask culture. Culture conditions have not yet been optimized for this relatively new cell type. In the example cited below, nearly 3 mg of antibody was produced over a four-week period of time. RPMI media with 10% CDM-HD was found to pro-

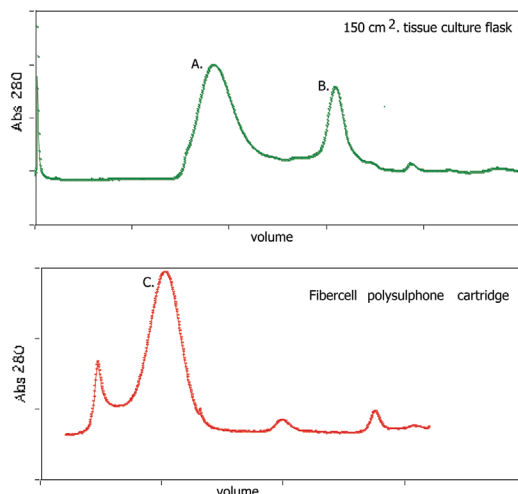
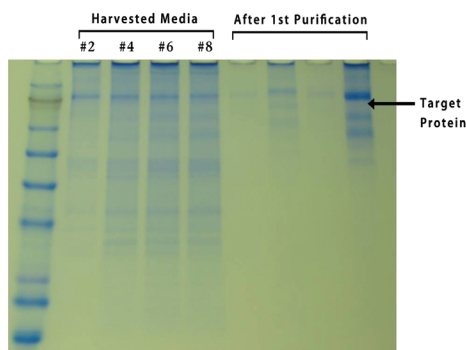


Figure 5 Comparison of a recombinant human hexamerized IgG produced from CHO cells grown in either a flask (upper panel) or a **FiberCell Systems** polysulfone cartridge (lower panel). Gel filtration chromatography reveals both a highly polymerized hexamerized IgG (peak A) and nonpolymerized (peak B); 476 mg of protein was produced in two months in a harvest volume of less than 5 L using **FiberCell Systems** cartridge C2018. (Data courtesy of Dr. Jim Arthos, Bethesda, MD.)

vide the best performance for this particular clone: Medium consumed—10 L, harvest volume—200 mL, total rabbit MAb produced—2.9 mL, average concentration—0.0145 mg/mL. (Data courtesy of Tong-Ming Fu and Daniel Freed, **Merck and Co.**, West Point, PA.)

### Case study 1: Recombinant protein

Purified recombinant protein (473 mg) from a Chinese hamster ovary (CHO) cell line was harvested from the C2018 (20 kd MWCO) cartridge (**FiberCell Systems**). Medium was DMEM with 2% fetal bovine serum (FBS); each harvest was 70 mL in volume, and total harvest volume was 4.8 L for an average concentration of approximately 100 µg/mL/day. The protein was a very complex hexamerized IgG consisting of six IgG1 subunits held together with three IgA tails. The Fv region was modified to contain CD4 receptor.<sup>3</sup> The cartridge consumed an average of 2 L of medium per day over a 60-day production period. Most interesting was the comparison of protein produced using T-flasks versus the hollow-fiber cartridge. When produced in flasks, approximately 40% of the protein was secreted as an unfolded mono-



**Figure 6** SDS-PAGE of unpurified, dialyzed harvest from a DG44 CHO cell line grown in the **FiberCell Systems** cartridge C2011 using serum-free, protein-free medium. The harvest is clean and nearly free of contaminating intracellular proteins, demonstrating low levels of apoptosis.

meric subunit. Placing the exact same cells into the hollow-fiber cartridge resulted in nearly 95% of the protein being produced as a properly folded hexamer. Improved cell culture conditions resulted in better protein expression fidelity (see *Figure 5*).

### Case study 2: Recombinant protein

Purified recombinant IgG1 (246 mg) from a CHO (DG44) cell line was harvested from the C2011 (20 kd MWCO) cartridge (**FiberCell Systems**). Medium was a serum-free, protein-free formulation similar to CDM-HD. Each harvest was 19 mL in volume; total harvest volume was 304 mL for an average concentration of over 800 µg/mL/day. The cartridge consumed 1 L of medium every two days, and the culture was maintained for a total of 35 days. Even though cell viability in the harvest was fairly low, the expressed protein was very clean, as demonstrated by the gel of the unpurified harvest shown in *Figure 6* and *Table 1*. Contamination with cellular proteins and DNA was quite low.

HFBRs allow laboratories to produce significant quantities of monoclonal antibodies, rabbit monoclonal antibodies, and recombinant proteins from CHO, 293, and other mammalian cell lines under serum-free conditions. The product is concentrated, free from contaminating serum and intracellular proteins and DNA, and

**Table 1 Growth of DG44 CHO cell line and production in 20-kd MWCO FiberCell cartridge**

Harvested samples	Target protein conc. (µg/mL)	Harvested volume	Total protein (mg)	Days	Used media (mL)
1	359.8	19	6.8	2	500
2	653.0	19	12.4	2	1000
3	1046.1	19	19.9	3	
4	1168.6	19	22.2	2	1000
5	984.8	19	18.7	2	1000
6	1257.0	19	23.0	3	
7	916.5	19	17.4	2	1000
8	1148.2	19	21.8	3	
9	937.0	19	17.8	2	1000
10	841.4	19	16.0	2	500
11	838.0	19	15.9	2	1000
12	646.1	19	12.3	2	
13	639.2	19	12.1	2	1000
14	494.7	19	9.4	2	1000
15	563.6	19	10.7	2	1000
16	484.3	19	9.2	2	
Total	811.1	304	246.6	35	10,000

may also have more uniform and complete post-translational modifications. These advantages also apply to secretome analysis from cancer cells for the study of biomarkers,<sup>4</sup> protein production from S2 cells, the generation of conditioned medium for stem cell culture support, and primary stem cell culture. Primary human placental-derived mesenchymal stem cells have been supported for more than three months of culture in a hollow-fiber cartridge (**FiberCell Systems**; data not shown).

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. Use of CDM-HD renders the medium used both economical and chemically defined/protein-free. Cultures can be maintained for long periods of time, meaning that scalability of the system is determined by length of culture, not new equipment. The advancement of higher-capacity HFBRs to a production-scale unit would represent a

potential paradigm shift in recombinant protein biomanufacturing.

### References

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Mr. Cadwell is President, **FiberCell® Systems, Inc.**, 905 W. 7th St. #334, Frederick, MD 21701, U.S.A.; tel.: 301-471-1269; e-mail: jcadwell@fibercellsystems.com.