FiberCell Systems

A BETTER WAY TO GROW CELLS

User Manual & Quick Start Guide

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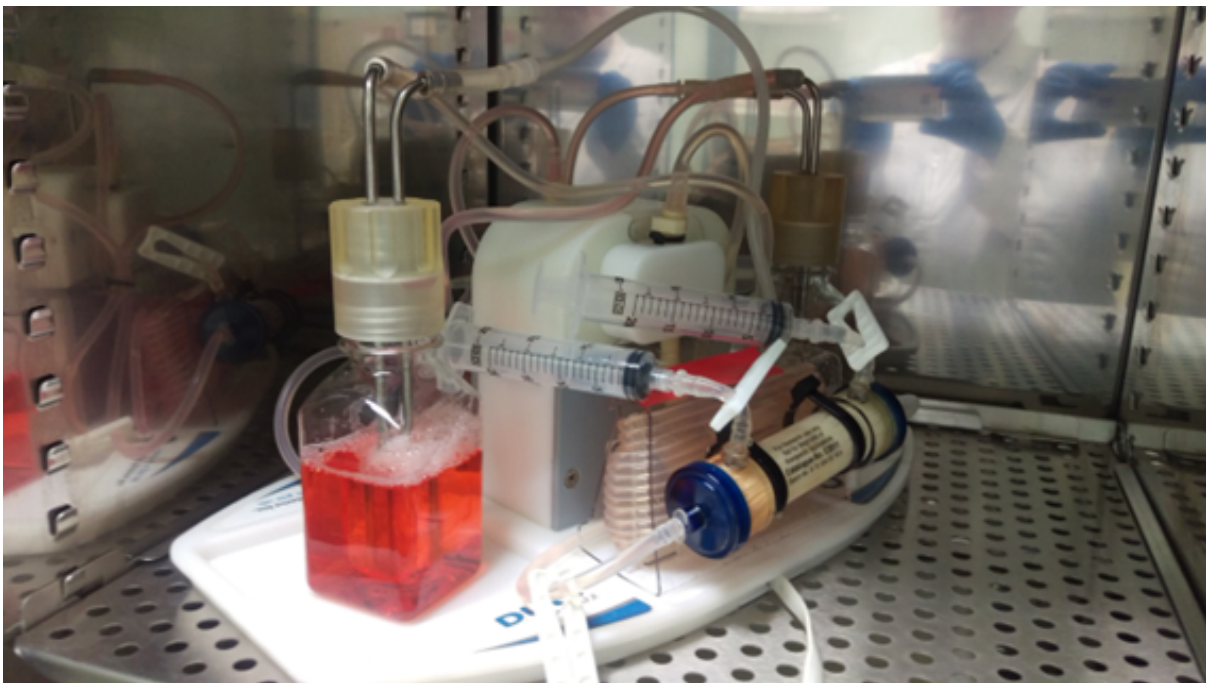

Figure 1: FiberCell Systems' 3D Hollow Fiber Bioreactor with Duet Pump in an Incubator.

1.0 Introduction

1.1 Overview

Section 1.0 (Introduction): Welcome to The FiberCell Systems User's Manual. This guide is designed to help you efficiently use our Hollow Fiber Bioreactor system. Above is a table of contents to help you navigate through the manual easily. This is the first revision of our full manual in over ten years and distills all our experience into one, easy-to-use document. To ensure safe and successful operation, we provide essential information on warnings, safety instructions, and definitions of terms here. Technical support is available to answer any questions you might have. You will also find valuable additional resources to further assist you in using the bioreactor effectively.

Section 2.0 (Theory of Hollow Fiber Bioreactors): This section gives you a comprehensive understanding of the Hollow Fiber Bioreactor, its theory, applications, and key steps. It also includes the FiberCell Systems Hollow Fiber Bioreactor Selection Guide and basic laboratory procedures.

Section 3.0 (Quick Start Guide): For a quick start, follow the step-by-step instructions in section 3. It covers setting up the cartridge, general culture guidelines, materials required, reservoir cap assembly, sterilization, pre-culture, cell inoculation, daily maintenance, and harvesting. In addition, you will find daily maintenance procedures to keep your bioreactor in top condition for optimal results. The calculation of glucose uptake rate is detailed in under this section, providing valuable insights into the performance of your bioreactor system.

Section 4.0 (Application Specific Guidelines): To cater to specific applications, section 4.0 presents guidelines for working with monoclonal antibodies, recombinant proteins, Extracellular Vesicles and Mesenchymal Stem Cells, Co-Cultivation and 3-D culture, and General Culture Guidelines For Other Cell Types.

Section 5.0 (Tips & Troubleshooting): The Tips and Troubleshooting section (5.0) offers helpful advice to address common issues and optimize your bioreactor's performance. The Hybridoma Troubleshooting Guide offers additional insights into the culture of hybridoma cells and production of monoclonal antibodies.

Section 6.0 (CDM HD Instruction): This section provides instructions for using CDM HD, and some tips on applications and purification.

Section 7.0 (Duet Pump Operating Instructions): This covers the operation of the Duet Pump system.

Section 8.0 (Appendix: Additional Lab Supplies and Vendors): Finally, in Appendix 1, you'll find a list of suppliers to source any additional materials you might need.

We hope this user-friendly manual enhances your experience with our Hollow Fiber Bioreactor system. If you need further assistance, feel free to contact our technical support team. Welcome to 3-D perfusion hollow fiber bioreactors!

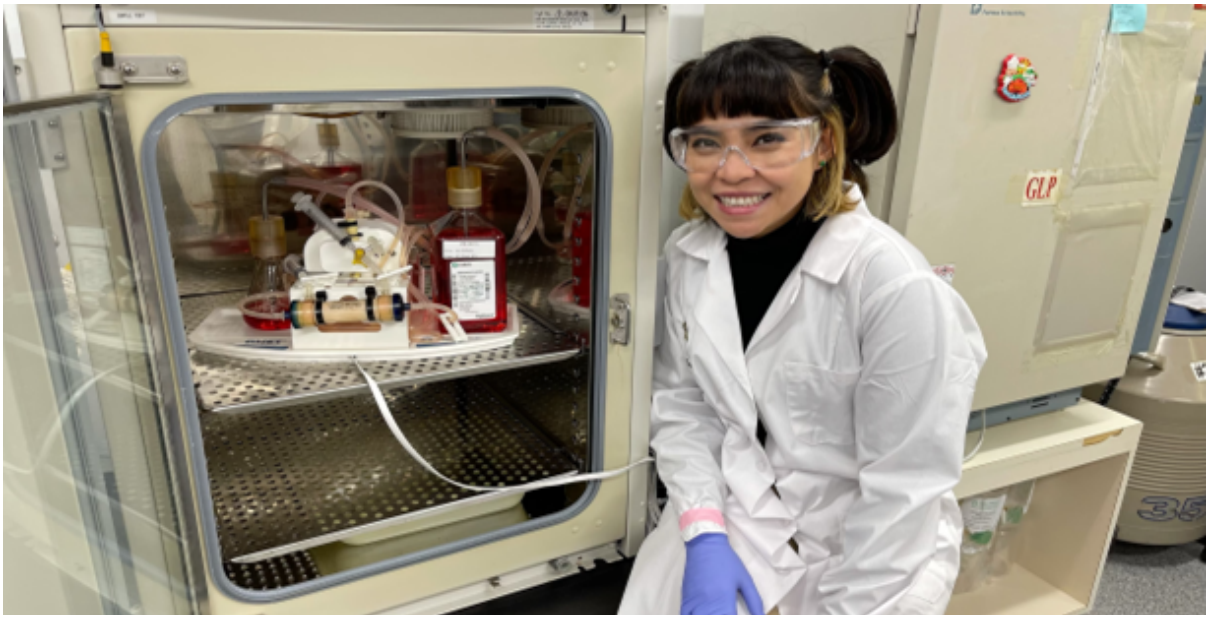

Figure 2: FiberCell Systems' 3D Hollow Fiber Bioreactor with Duet Pump in an Incubator.

1.2 Warning and Safety Instructions

To ensure safe and efficient operation of the FiberCell Duet, please follow these important safety guidelines:

Electrical

- The FiberCell Duet operates on 24-volt DC, and the universal power supply accommodates various voltage inputs.
- For your safety, never attempt to service the Transformer, Control Box, or Perfusion Pump. Contact our authorized service center for any maintenance or repairs.
- Avoid placing the Transformer or the Control Box inside the incubator to prevent potential hazards.

Cleaning and Disinfecting

- If necessary, you can clean the Transformer, Control Box, and Perfusion Pump using mild soap and warm water.
- Never immerse any of these components in water, as it may cause damage.
- Avoid using organic solvents or corrosive chemicals for cleaning or disinfecting purposes, as it may affect the functionality and safety of the equipment.
- Steam sterilization or heat should not be used for disinfection.

Safety

- When working with the FiberCell module, always adhere to the biosafety levels recommended for the infectious agents you handle. After use, dispose of it according to local Health Safety Guidelines to prevent any potential risks.
- For personal protection, it is essential to wear lab coats and gloves whenever you handle the FiberCell module.

We prioritize your safety and the reliable performance of our products. By following these guidelines, you can ensure a secure and efficient experience with the FiberCell Duet. If you have any questions or concerns, please feel free to contact our customer support team at **info@fiber-cellsystems.com** or call us at **301-471-1269**. All FiberCell Systems products come with a complete 2-year warranty.

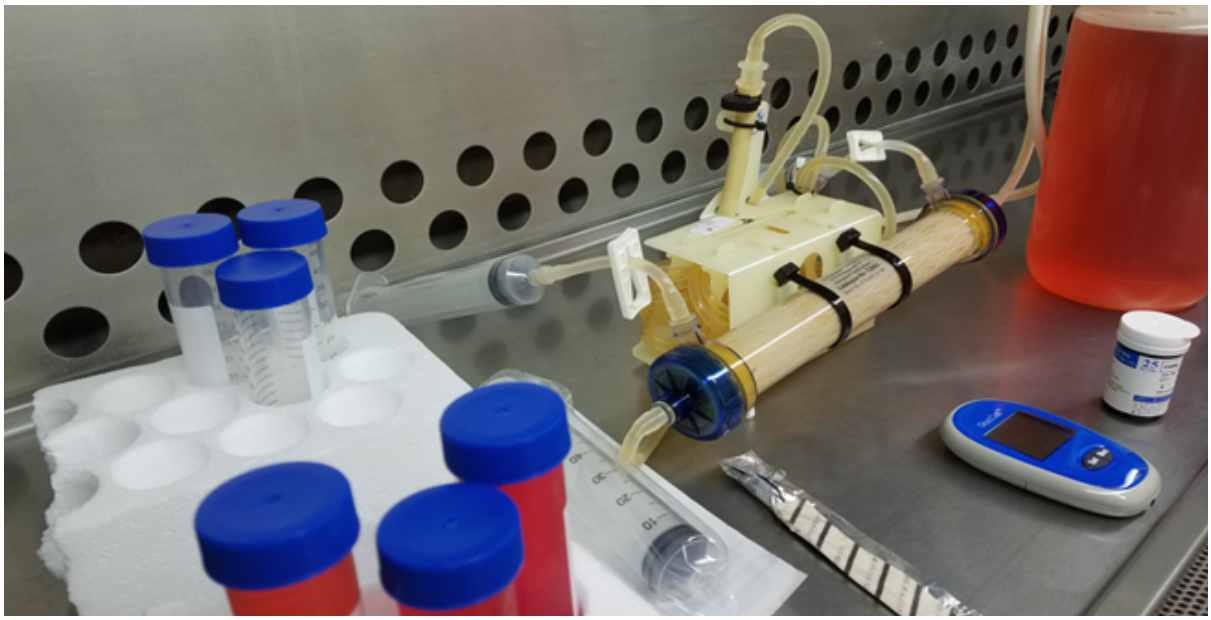

Figure 3: FiberCell Systems' 3D Bioreactor (C2018 Large Cartridge) with Harvests.

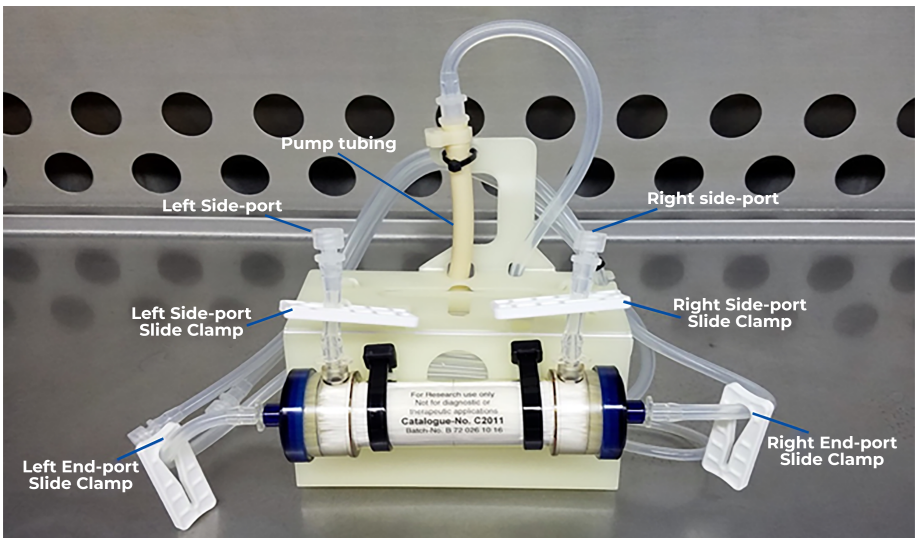

Figure 4: C2011 medium cartridge with parts identified.

1.3 Glossary: definition of terms:

To help you understand the key concepts related to the FiberCell Duet, we have provided the following definitions:

- **Dalton:** The mass of a hydrogen atom. This unit is used to define molecular weight cut-offs, which are crucial in the separation of molecules based on their size.
- **ECS (Extracapillary Space):** This refers to the volume inside the cartridge housing and outside the fiber bundle. The ECS is separate from the ICS (Intracapillary Space) by the hollow fiber filter.
- **ICS (Intracapillary Space):** The volume inside the fibers of the cartridge, where the process of filtration and separation occurs.
- **End Port:** Left and Right. Connections on the end of the cartridges that allow medium to flow through the insides of the fibers, facilitating the exchange of substances. Medium enters the left end port and exits through the right end port.
- **Side Port:** Left and Right. Ports located on the sides of the cartridge with tubing and luer connections, providing access to the contents of the ECS for sampling or other purposes.
- **Pump Tubing:** Brown tubing on the flow path stand that is compressed by the Duet pump piston to create flow within the system.
- **Oxygenator Tubing:** A loop of tubing wrapped around the back of the flow path stand, designed for passive gas exchange to maintain proper oxygen and carbon dioxide levels.
- **Flow Path Stand:** A plastic stand that securely holds the cartridge in place, provides space for the loop of oxygenator tubing, and ensures the pump tubing is correctly positioned for optimal flow.
- **Pump Piston:** The piston within the Duet that applies pressure to the pump tubing, generating the necessary flow for your experiments.
- **MWCO (Molecular Weight Cut-Off):** This term refers to the lowest molecular weight solute, measured in Daltons, that determines the size of molecules allowed to pass through the membrane. For example, a globular protein with a molecular weight of 90% retained by the membrane is considered to have an MWCO of that value. Note that MWCO can also be defined as the molecular weight at which 90% of the analytes or solutes are prohibited from membrane diffusion.

We hope these definitions make it easier for you to work with the FiberCell bioreactor modules and understand the essential terms related to their operation. If you have any further questions or require clarification, feel free

to reach out to our customer support team at **info@fibercellsystems.com** or call us at **301-471-1269.**

Figure 5: FiberCell Systems's 3D Bioreactor (C2011 Medium Cartridge) with Media Bottle.

1.4 Technical Support

Over the past 30 years, the culture of over 200 different cell lines in hollow fiber bioreactors has led to the refinement of general systems and protocols. This evolution has made hollow fiber cell culture a straightforward and reliable technology.

At FiberCell, we offer comprehensive protocols and application guides for all common applications of hollow fiber bioreactors. Whether you are interested in producing secreted proteins, exosomes, 3-D cell culture models, or other applications, our protocols can assist you in achieving optimal results. We pride ourselves in our interactive technical support. Lifetime support and protocol development is the cornerstone of FiberCell systems. If you need specific protocol development for your unique application, we are here to help. Please feel free to contact us at **info@fibercellsystems.com** or call us at **301-471-1269**. Our experienced team will be glad to provide you with expert assistance and support to ensure successful implementation of hollow fiber bioreactors in your research or biopharmaceutical processes.

1.5 Additional Resources

To further enhance your experience with the FiberCell Duet, we offer a variety of additional resources to support your research and provide valuable insights:

- **FiberCell Video Instructions:** In addition to this user manual, we have video instructions available to guide you through the setup and operation of the FiberCell Duet. You can access these helpful videos by visiting **https://www.youtube.com/@fibercellsystems**.

- **White Papers and Posters:** Our FiberCell website hosts a collection of informative white papers and posters that delve deeper into specific topics related to hollow fiber bioreactors. These resources can offer valuable scientific background and practical tips. To access them, please visit **https://www.fibercellsystems.com/technical-resources/technical-documents**.

- **Literature References:** The scientific community has widely adopted the FiberCell Duet, and numerous publications highlight its applications and results. On our website, you can find a comprehensive list of references and research papers that showcase the versatility and effectiveness of the FiberCell Duet. To explore these publications, please visit **https://www.fibercellsystems.com/technical-resources/articles/**.

By utilizing these additional resources in combination with this user-friendly manual, you'll gain a thorough understanding of the FiberCell Duet's capabilities and maximize your experimental success. Should you require any further assistance or have any questions, our dedicated customer support team at **info@fibercellsystems.com** or call us at **301-471-1269.** We are always available and happy to help.

2.0 Theory of Hollow Fiber Bioreactors

2.1 Background

2-D cell culture: In the early days of tissue culture, cells were cultured on porous supports, such as plasma clots, or non-porous supports like glass or plastic culture dishes. Fetal bovine serum played a significant role in cell culture by providing attachment factors and essential growth factors to supplement the basal medium.

Traditional static culture methods result in a non-physiologic, dynamic environment for cells, characterized by fluctuating conditions:

- Lag Phase: Cells condition the medium, preparing it for growth.
- Exponential Phase: Cells experience maximum growth and viability.
- Stationary Phase: Medium nutrients are depleted, leading to decreased metabolic activity and viability.
- Death Phase: Conditions like decreased oxygen, high lactic acid, ammonia levels, and decreased pH contribute to cell death.

To maintain the cultures, cells must be transferred to new flasks with fresh medium periodically, perpetuating the cycle. However, there is only a brief

window during the exponential phase when cell culture conditions are truly physiological and optimal.

Although 2-D culture has been accepted by the scientific community it is becoming increasingly clear that it is wasteful, time consuming and produces results that are not necessarily reflective of the in vivo cellular physiology.

Introduction to 3-D Hollow Fiber Bioreactors

Hollow fiber bioreactors offer an innovative solution to overcome the limitations of traditional static culture methods. These bioreactors provide a controlled and continuous culture environment that closely mimics in vivo conditions, resulting in more physiologically relevant cell behavior and improved productivity.

Key Advantages of Hollow Fiber Bioreactors:

- **Continuous Culture:** Hollow fiber bioreactors enable continuous culture, maintaining cells in their exponential growth phase for extended periods. This approach eliminates the feast or famine environment of static cultures and provides a consistent supply of nutrients, maximizing cell viability and productivity. The world record for continuous culture of a glioma cell line in our hollow fiber bioreactor is over 2 years of continuous culture. This varies by cell type or application.

- **Physiological Relevance:** The three-dimensional configuration of hollow fiber bioreactors better simulates the in vivo microenvironment, promoting more accurate cell behavior and maintaining cell differentiation and function.

- **High Cell Density:** Hollow fiber bioreactors allow for higher cell densities compared to traditional static cultures, leading to increased production of desired products, such as monoclonal antibodies, recombinant proteins, exosomes, and more.

- **Easy Sampling and Medium Exchange:** With access to side ports on the bioreactor, researchers can easily sample the extracapillary space (ECS) and exchange medium as needed without disturbing the cells within the fibers.

- **Long-Term Cultures:** Hollow fiber bioreactors support long-term cultures, reducing the need for frequent cell passages and optimizing experimental timelines.

For in-depth insights and application-specific information, we encourage you to explore the various webinars available on our website at **www.fibercellsystems.com.** These webinars provide valuable knowledge on how hollow fiber bioreactors can revolutionize your cell culture experiments and drive your research forward.

Key Disadvantages of 2-D Flask Culture

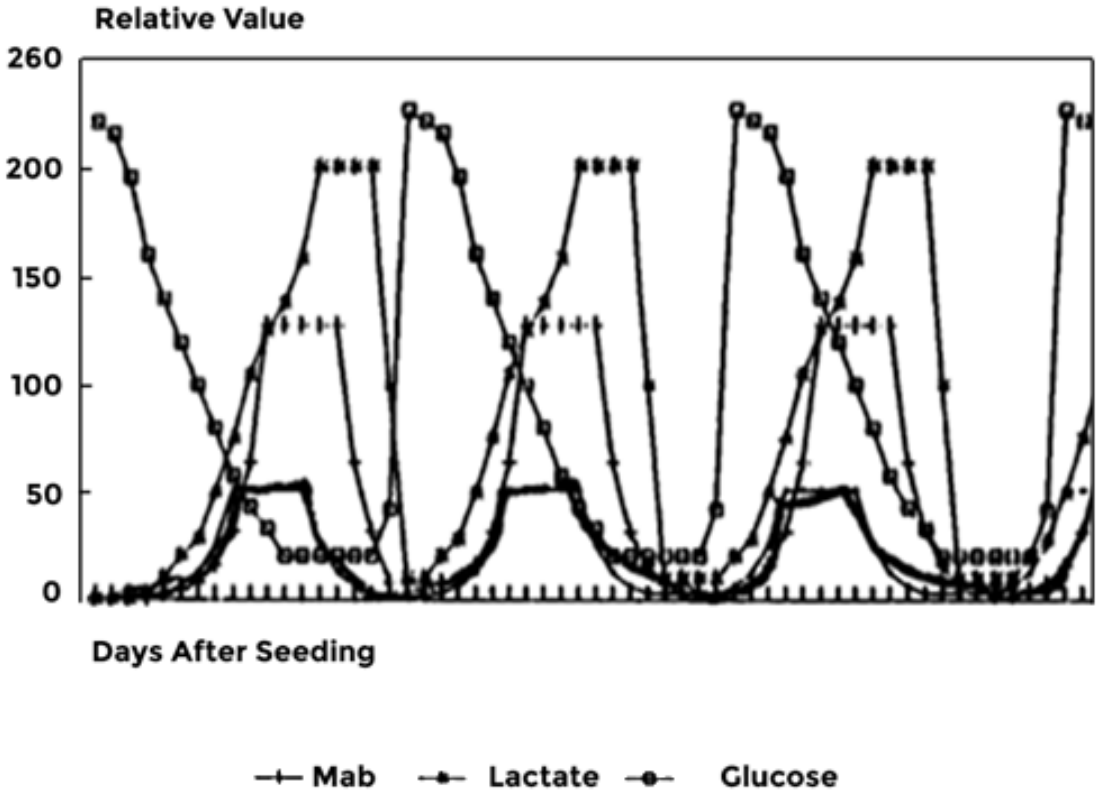

Figure 6: Feast or Famine Cycle for Hybridoma Cells in a Static 2-D Flask

In this cycle, hybridoma cells cultured in a static flask go through distinct phases:

- **Lag Phase:** After inoculation, the cells take some time to adapt to the environment and condition the growth medium. Minimal growth occurs during this phase.

- **Log Phase Growth:** Following the lag phase, cells enter the log phase of growth. During this stage, cell proliferation accelerates, leading to increased glucose consumption. As cells metabolize glucose, they produce lactic acid and other waste products. The accumulation of lactic acid results in a rapid drop in the medium's pH, making it more acidic.

- **Split or Passaging:** When cells reach confluence in the flask they must be split or passaged into new flasks with fresh medium. They must be maintained as a sub-confluent, monolayer culture.

Challenges: This Feast or Famine cycle demonstrates the challenges of traditional static cultures. Fluctuations in nutrient availability and waste product buildup are non-physiologic and create suboptimal conditions and limit the window of optimal cell growth.

Solution: To address these limitations and maintain a more stable and continuous culture environment, 3-D perfusion hollow fiber bioreactors were developed. These bioreactors support continuous culture, minimizing nutrient fluctuations and waste product buildup, resulting in improved productivity and more physiologically relevant cell behavior.

Non-Physiologic 2-D: Classical batch-style 2-D cultures on non-porous supports in flasks or low-density suspension cultures in spinners or shakers are limited in their biological relevance. These culture methods may not accurately represent how cells behave in their natural in vivo environment, and the conditions can impact the quality and purity of secreted products.

Physiologic 3-D: The hollow fiber bioreactor offers a high-density, continuous perfusion culture system that closely approximates the in vivo cell growth environment. The bioreactor consists of thousands of semi-permeable hollow fibers arranged in a parallel array within a cartridge shell fitted with inlet and outlet ports. These hollow fiber bundles are sealed at each end, ensuring that any liquid entering the cartridge flows through the interior of the fibers.

Cells are typically seeded outside the fibers within the cartridge, in what is known as the extra-capillary space (ECS). In this setup, the hollow fiber bioreactor incorporates all the essential support systems found in the vertebrate body. It includes the organ (bioreactor), blood (the circulating medium), lung (oxygenation system), capillary bed (hollow fibers), and heart (pulsatile perfusion pump).

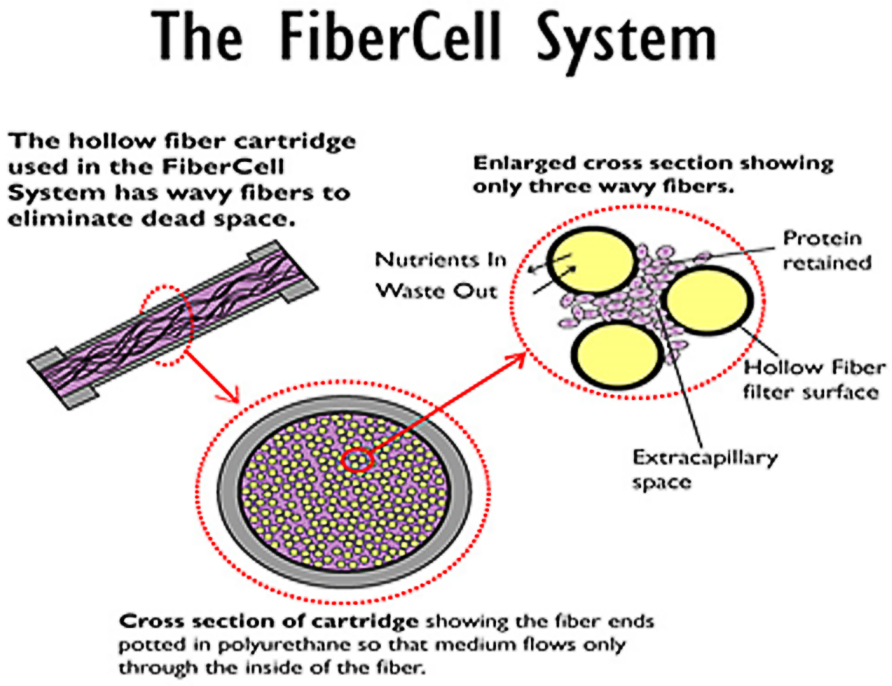

Figure 7: Diagram of a hollow fiber bioreactor

With its advanced design, the hollow fiber bioreactor provides a more physiologically relevant and controlled culture environment, allowing for continuous and high-density cell growth. This mimics the natural in vivo conditions, leading to improved cell behavior and enhanced productivity for a wide range of applications, including the production of secreted products like monoclonal antibodies, recombinant proteins, and exosomes.

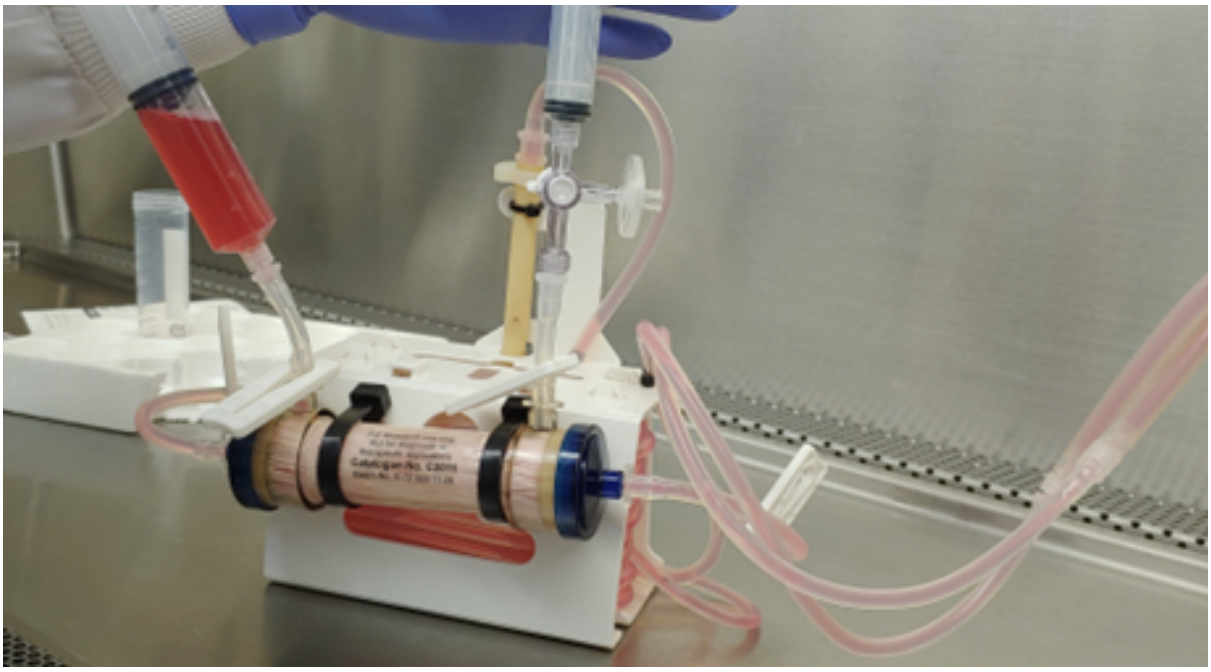

Figure 8: Harvests are being collected from the cartridge.

A simple overview of how the hollow fiber bioreactor works:

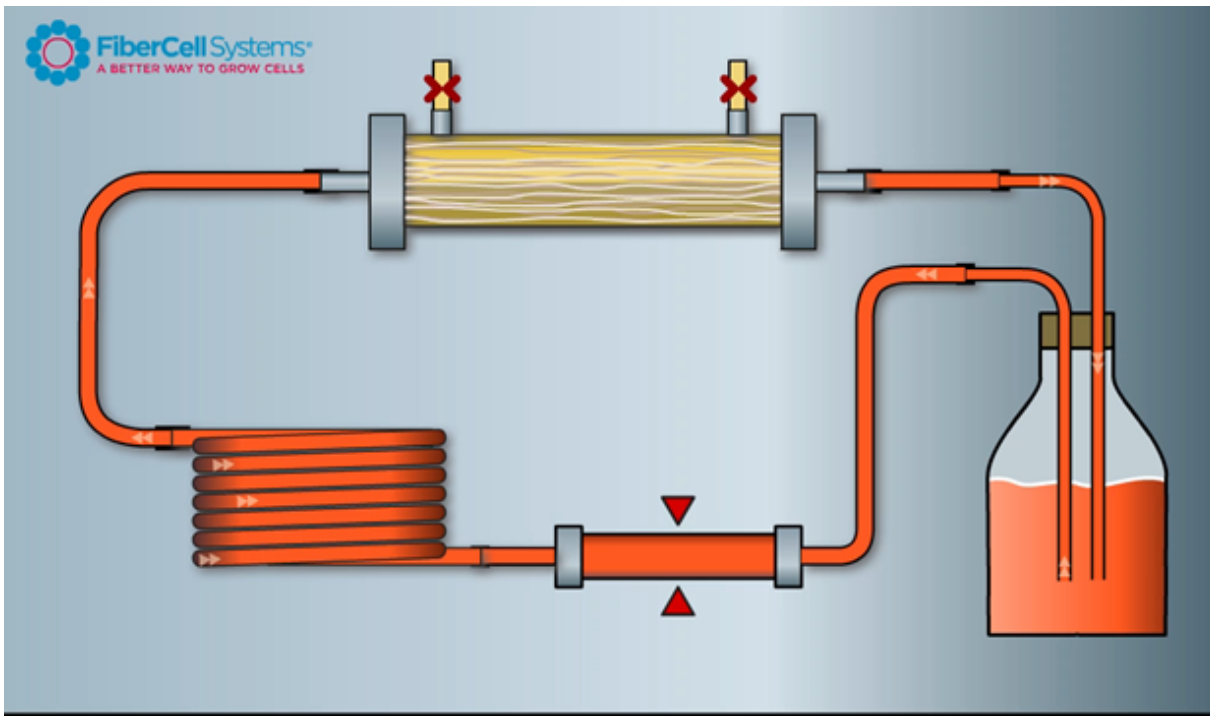

Figure 9: Schematic of a Hollow Fiber Bioreactor:

- **Medium Flow:** The culture medium is pumped from the reservoir bottle using a pulsatile perfusion pump. It then travels through the oxygenation circuit, where it gets oxygenated and saturated with CO2 to support optimal cell growth.

- **Cell Growth Environment:** Within the bioreactor, cells grow in a 3-dimensional environment. They are surrounded by a continuous flow of fresh medium that perfuses through the hollow fibers, providing them with essential nutrients for continuous high-density growth.

- **Waste Removal:** As cells metabolize nutrients, waste products are produced. The hollow fibers serve as a dialysis system, removing these metabolic waste products from the cells. The waste is then diluted into the circulating medium to maintain a clean and controlled environment.

The hollow fiber bioreactor provides a more physiologically relevant culture environment, mimicking how cells grow in vivo. This continuous and high-density culture system enhances cell physiology and productivity, making it a valuable tool for various applications, including the production of secreted products. The 3-D Hollow Fiber Bioreactor is shown below, in cross-section.

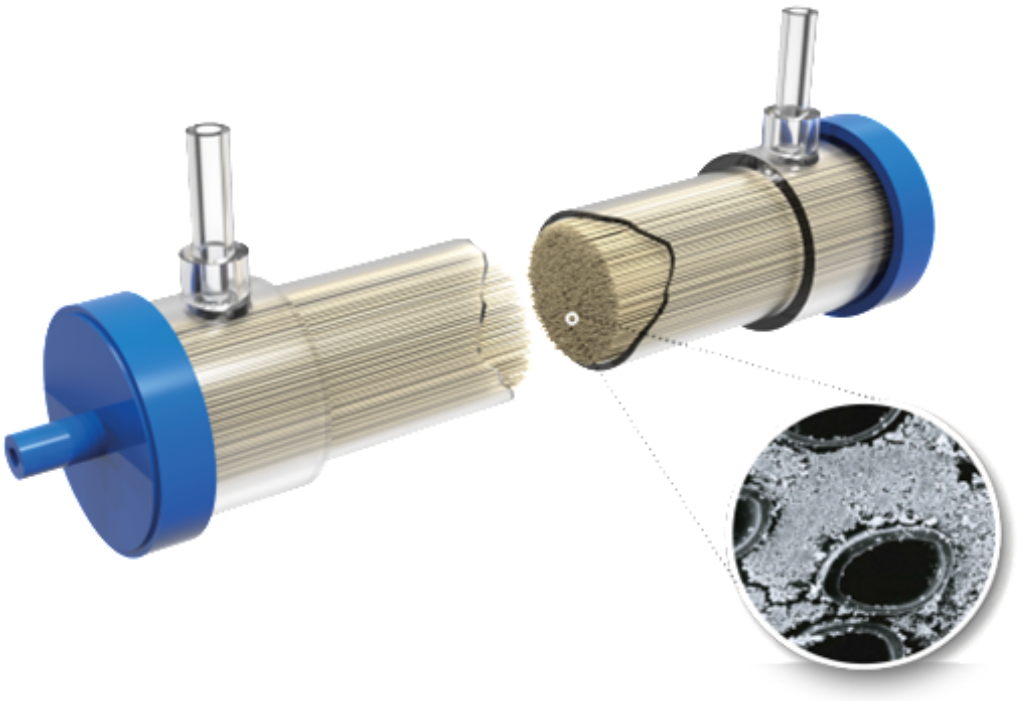

Figure 10: Hollow fiber bioreactor in cross-section.

Key processes:

- **Medium Perfusion:** The medium flows through the lumen of the hollow fibers, delivering vital oxygen and nutrients directly to the cells. This continuous supply of fresh medium sustains optimal cell growth.

- **Waste Removal:** As cells metabolize nutrients, waste products like lactic acid and ammonia are produced. The hollow fibers act as a dialysis system, removing these metabolic waste products away from the cells. The waste is then diluted into the medium contained in the reservoir bottle, maintaining a clean and controlled environment for the cells.

- **3-Dimensional Growth Environment:** The cells grow in a 3-dimensional environment within the bioreactor, allowing them to establish numerous physical contact points with neighboring cells. This setup promotes a more natural and physiologically relevant cell behavior.

- **Secretion of Factors:** Cells secrete various autocrine and paracrine factors that accumulate within the extra capillary space (ECS). This localized accumulation of factors creates a unique microenvironment, contributing to improved cell function and productivity.

The high cell density in the hollow fiber bioreactor fosters a self-sustaining microenvironment, enabling cells to thrive and perform optimally. This system's continuous and high-density culture approach makes it ideal for various applications, such as producing secreted products like monoclonal antibodies, recombinant proteins, and exosomes. **The hollow fiber bioreactor provides a powerful tool for researchers to achieve more biologically relevant results and advance their scientific studies.**

Key Benefits of Hollow Fiber Bioreactors:

- **In Vivo-Like Cell Attachment:** Cells are bound to a porous matrix, closely mimicking their natural in vivo environment, unlike traditional culture methods on plastic dishes or microcarriers. In this perfusion system, cells maintain viability and relevant metabolism for extended periods, without the need for frequent cell splitting. Passage number becomes irrelevant, and cells can exhibit prolonged productivity and reduced apoptosis, resulting in a cleaner and easier-to-purify product.

- **Controlled Molecular Weight Cut-Off:** The fiber's molecular weight cut-off can be precisely controlled, enabling the retention of desired products at higher concentrations and the selective removal of inhibitory cytokines. For instance, in hybridoma culture, the retention of secreted antibodies and cells while removing inhibitory cytokine TGF beta is achievable.

- **High Surface Area to Volume Ratio:** Hollow fibers' small diameter generates an exceptionally high surface area to volume ratio, approximately 100-200cm^2 per ml. This, combined with FiberCell's high gross filtration rate, facilitates rapid nutrient and waste product exchange. The resulting high cell densities, close to in vivo tissue-like densities, lead to increased protein production per milliliter volume. A small 20mls

cartridge can support as many cells as a large 2-liter spinner flask or 40 or more roller bottles. High cell densities also allow for easier adaptation to lower serum concentrations or protein-free serum replacements like CDM HD (**C**hemically **D**efined **M**edium for **H**igh **D**ensity culture) leading to cleaner product harvests and simplified purification processes.

By leveraging these three fundamental characteristics, hollow fiber bioreactors offer a more biologically relevant and efficient cell culture system, making them valuable tools for a wide range of applications, including monoclonal antibody production, recombinant protein expression, and other biopharmaceutical processes.

Other Benefits:

Hollow fiber cell culture offers a transformative approach to cell culture, presenting several key advantages over conventional flask and spinner cultures:

- **Long-Term Culture with In Vivo-Like Conditions:** Cells are bound to a porous support, eliminating the need for frequent splitting. Passage number becomes irrelevant, enabling cultures to be maintained for extended periods, even up to many months, under more in vivo-like conditions.

- **High Cell Densities:** Hollow fiber bioreactors support high cell densities, typically 1-2X108 cells per milliliter. This high density is closer to physiologic levels and allows cells to generate their own specific microenvironments.

- **Optimization with CDM HD:** CDM HD, a near-universal, protein-free, and chemically defined serum replacement, is specifically designed for high-density cell culture, as found in hollow fiber bioreactors. It offers specific micro-nutrients, amino acids, and additional buffering capacity without surfactants.

- **Significantly Higher Product Concentrations:** Hollow fiber bioreactors concentrate secreted products, such as proteins and antibodies, up to 100 times higher than in flask or spinner cultures. This leads to cleaner and more concentrated harvests.

- **Enhanced Post-Translational Modifications:** The more in vivo-like culture conditions in hollow fiber bioreactors can result in improved protein folding and more uniform glycosylation patterns over time.

- **Reduced Apoptosis and Contamination:** Hollow fiber cultures can significantly reduce apoptosis, resulting in lower contamination with intracellular DNA and proteins, leading to purer products.

- **Removal of Inhibitory Cytokines:** Inhibitory cytokines can be dialyzed

away from the cells, allowing for improved cell growth and productivity.

- **Surfactant-Free Medium:** The medium used in hollow fiber bioreactors does not require surfactants, simplifying the culture process.

Hollow fiber cell culture has the unique ability to maintain cells at high densities, closely resembling in vivo conditions.

2.2 Applications

Hollow fiber bioreactors offer a wide range of applications in cell culture, including:

- **Monoclonal Antibody Production:** Hollow fiber bioreactors are the ideal method to produce milligrams on up to gram quantities of a monoclonal antibody. The MWCO of 20kDa allows the inhibitory cytokine TGF-beta to diffuse away from the cells while the cells and secreted antibody remained trapped to high concentrations (.5 mg/ml to 5 mgs/mls) in the ECS of the cartridge. Typical productivity is 10-100 mgs every two days.

Figure 11: Production of Recombinant Proteins in 3D Hollow Fiber Bioreactors.

- **Recombinant Protein Production:** Hollow fibers are ideal for producing recombinant-proteins, and conditioned medium. The continuous perfusion system promotes high-density cell growth and enhanced protein production with complete and uniform post-translational modifications. Productivity is between 1-20 mgs per day.

- **Extracellular Vesicles Production:** Hollow fiber bioreactors support the culture of cells for exosome production. Exosomes are small extracellular vesicles that play essential roles in cell-to-cell communication and are of great interest in research and therapeutics.

- **3-D Culture and Co-Cultivation:** Hollow fibers enable co-cultivation of two cell lines, often involving feeder cells and expansion cell lines. This setup facilitates complex cellular interactions and creates specialized microenvironments for specific applications.

- **Endothelial Cell (EC) and EC/Smooth Muscle (SMC) Culture:** Hollow fiber bioreactors are used for culturing EC and EC/SMC under controlled shear stress and pulsatile perfusion conditions. These models help study blood vessel physiology and vascular tissue engineering.

- **EC/Astrocyte Blood Brain Barrier (BBB) Models:** Hollow fiber bioreactors support the creation of EC/astrocyte BBB models, crucial for studying drug transport across the blood-brain barrier and neurological diseases.

- **Bio-Artificial Liver (BAL) Devices:** Hollow fiber bioreactors are employed in bio-artificial liver devices, facilitating liver cell culture and metabolic studies.

- **Cell Culture on Specific Extracellular Matrices:** Researchers can culture specific cell types on specialized extracellular matrices using hollow fiber bioreactors, creating tailored environments for various applications.

- **Parasite Culture:** Several different disease-causing parasites have been cultured in the 3-D hollow fiber bioreactor including cryptosporidium and plasmodium sporozoites.

- **Antibiotic Pk/Pd:** Hollow fiber bioreactors are valuable tools for conducting Pk/Pd studies to assess the toxicology and efficacy of drugs or compounds in vitro.

These diverse applications showcase the versatility and effectiveness of hollow fiber bioreactors in providing more physiologically relevant culture conditions, high-density cell growth, and improved productivity for a wide range of research and biopharmaceutical processes.

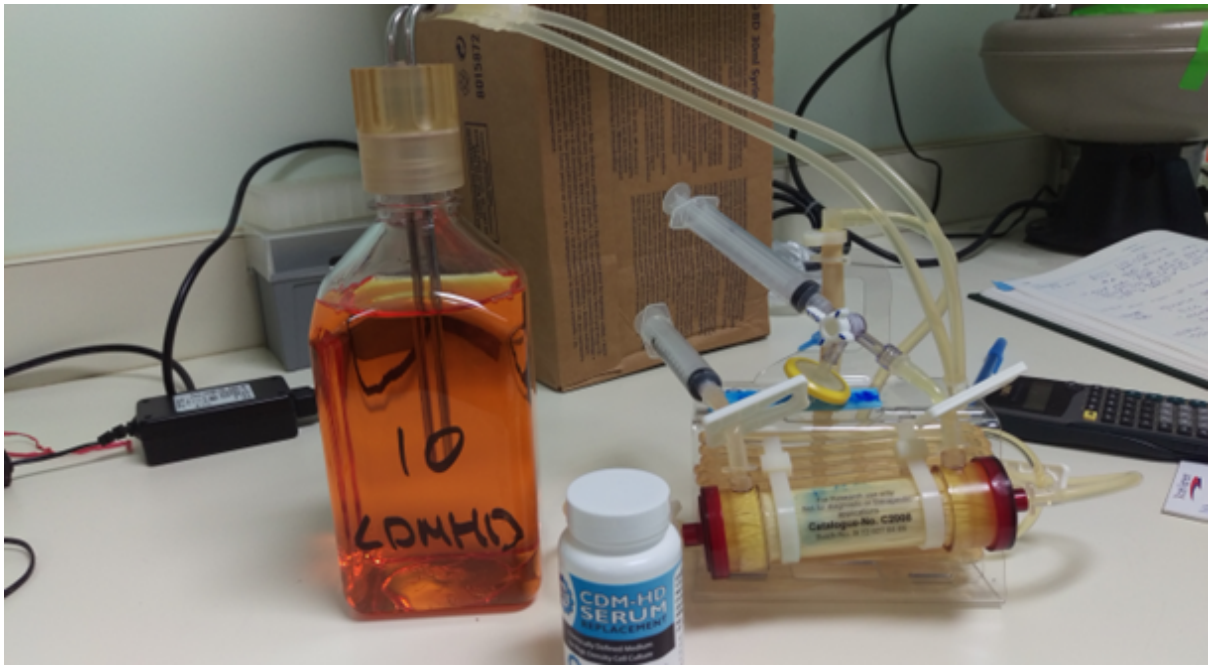

Figure 12: FiberCell System's Chemically Defined Serum Replacement for High-Density Culture (CDM HD).

2.3 Key Steps

The successful use of hollow fiber bioreactors involves the following key steps:

- **Preparation:** Gather all necessary materials and equipment for the experiment and ensure proper sterilization.
- **Selection of Fiber Type and MWCO:** Choose the appropriate hollow fiber type and molecular weight cut-off (MWCO) based on the specific cell culture requirements.
- **Assembly of the Bioreactor:** Assemble the hollow fiber bioreactor, ensuring all components are securely connected.
- **Pre-culture with Growth Medium:** Condition the bioreactor with growth medium to create an optimal environment for cell growth.
- **Expansion of Cell Line(s) for Inoculation:** Expand the cell line(s) in a suitable culture vessel to reach the desired cell density for inoculation.
- **Harvest Cells for Inoculation:** Harvest the cells at their desired density for inoculation into the bioreactor.
- **Inoculation:** Introduce the harvested cells into the bioreactor to initiate the culture process.
- **Cell Attachment and Adaptation:** Allow the cells to attach and adapt to the bioreactor environment for optimal growth.
- **Production:** Promote continuous cell growth and proliferation within the bioreactor's 3-dimensional environment.
- **Monitoring Glucose in the Medium:** Regularly monitor glucose levels in the medium to ensure sufficient nutrient supply for cells.
- **Growth Rate Calculations:** Calculate the cell growth rate based on glucose consumption and other relevant parameters.
- **Correlation between Glucose Rates and Cellular Growth Rate:** Analyze the correlation between glucose consumption rates and cellular growth to optimize culture conditions.
- **Feeding and Medium Management Procedures:** Implement feeding and medium management procedures as needed to maintain optimal cell growth and productivity.
- **Harvest:** Harvest the cells or the secreted proteins/other products from the extracapillary space (ECS) or reservoir for further analysis or downstream applications.

By following these key steps, researchers can maximize the productivity and quality of their cell culture experiments using hollow fiber bioreactors.

2.4 Hollow Fiber Selection Guide For Cell Culture

2.4.1 Pore Size and Molecular Weight Cutoff (MWCO)

When considering the MWCO specifications of hollow fiber bioreactors, it's essential to understand that different manufacturers may use both the 50% and 95% levels for defining MWCO. There is alogarithmic relationship between molecular weight and percent retention. A Dalton is defined as the mass of a hydrogen atom.

For instance, a fiber with a 20kDa MWCO at 50% will retain approximately 50% of a secreted protein with a molecular weight of 20 kDa within the extracapillary space (ECS). However, the 95% value for the same fiber will be around 120 kDa, meaning it will retain approximately 95% of a secreted protein with a molecular weight of 100 kDa in the ECS.

If your secreted protein of interest falls within the range of 20 kDa to 100 kDa, the 5 kDa fiber is recommended. For proteins smaller than 20 kDa, consider using the 20kDa fiber and harvest the culture from the reservoir bottle. The higher gross filtration rate (GFR) of the 20 kDa fiber will result in more rapid cell growth due to faster exchange of nutrients and waste products.

However, it's essential to keep in mind that protein size is not solely defined by molecular weight but also influenced by glycosylation and shape, which can affect the protein's Stokes radius. Therefore, choosing the appropriate pore size and MWCO based on your specific protein size and requirements will ensure optimal performance and results in hollow fiber bioreactors. If you need further assistance or have specific questions about MWCO and its impact on your application, feel free to reach out to our customer technical support team at **info@fibercellsystems.com and +1 301 471 1269** for personalized support and guidance.

2.4.2 Criteria for Fiber Selection

The selection of fiber composition and pore size in hollow fiber bioreactors depends on various factors tailored to your specific application:

- **Anchorage Dependent Cells:** For cells that require attachment factors or serum for adhesion, the polysulfone fiber is suitable. Pre-culture with serum will generally provide sufficient attachment factors. Most proteins will bind to the surface by hydrophobic interaction. Specific matrix proteins such as fibronectin may also be used. There is no concern of fiber clogging by matrix proteins or serum.

- **Suspension Cells:** The hollow fiber bioreactor is also compatible with suspension cells. The fibers are cast with little "waves" so they press against one another to distribute the fiber bundle evenly within the housing. Many "suspension" cells will in fact bind to the fiber. This is to be desired as it means that the cells will grow at higher density and harvesting will preferentially remove the dead cells.

- **Secreted Products:** If you aim to produce secreted proteins, the pore size selection depends on the protein's size and whether it can be retained within the extracapillary space (ECS). Proteins larger than 80 kDa can be trapped within the ECS of the 20 kDa fiber. For proteins between 20kDa and 10kDa, the 5kDa fiber is appropriate.

- **Cytostatic or Cytotoxic Secreted Products:** If your cells secrete cytostatic or cytotoxic factors, select a molecular weight cut-off (MWCO) that allows these factors to diffuse away from the cells into the reservoir bottle. For example, TGF Beta secreted by hybridoma cells (MW 27 kDa) requires an appropriate MWCO. Frequent flushing of the ECS by harvesting can also control their effects.

- **3-D Co-Cultivation:** If co-cultivation is desired, consider the MWCO depending on the specific application. For stromal co-cultivation, the 5 kDa MWCO is typically used to concentrate secreted cytokines, while the 20 kDa fiber is also suitable. For endothelial cells, the PVDF fiber (catalog #C2025) is recommended.

Selecting the appropriate fiber composition and pore size ensures the ideal microenvironment for your cells, supporting their growth, productivity, and interaction for successful applications in hollow fiber bioreactors. If you need assistance with the selection process or have specific requirements, feel free to reach out to our technical support team at **info@fibercellsystems.com** and **+1 301 471 1269.**

Table I. FiberCell Cartridge Specifications

CATALOG NUMBER	MWCO (@50%)	AREA cm^2	FIBER TYPE	ECS VOL (ml)	GFR#	INNOCULATE# #
C2025	.1µm	$70cm^2$	PVDF	2	NA	$1\text{-}5X10^6$
C2025D	20 kDa	$450cm^2$	Polysulfone	2.8	300	$1\text{-}5X10^7$
C2011	20 kDa	4000 cm^2	Polysulfone	20	300	$1X10^8$
C2008	5 kDa	4000 cm^2	Polysulfone	20	12	$1X10^8$
C5011	20 kDa	$4000cm^2$	Polysulfone	20	300	$1X10e^8$
C2018	20 kDa	1.2 m^2	Polysulfone	70	300	$1\text{-}5X10^9$
C2003	5 kDa	1.2 m^2	Polysulfone	70	12	$5X10^9$

NOTES:
GFR# units: mls/mm Hg/cm2/hr
INNOCULATE# # or a number of adherent cells that will provide ~50% fiber surface confluence.

2.4.3 FiberCell Systems Cartridge Selection Guide

Here is a summary of the different hollow fiber bioreactor cartridges available, along with their recommended applications and capabilities:

▪C2011:

- Protein Range: 1 kDa - 10 kDa, greater than 80 kDa
- Recommended for: Hybridoma culture, recombinant protein production, exosome production, and general cell culture applications.
- MWCO: 20 kDa, allowing diffusion of TGF-beta and TNF-alpha while retaining antibodies and proteins greater than 80 kDa.
- Capacity: Can support 1-2X109 cells.
- Protein Production: 5-50 mgs of monoclonal antibody every two days, 2-5 mgs of a recombinant protein every day.
- Medium Consumption: Up to 1 liter of medium per day.

▪C2025D:

- 10X smaller version of the C2011 for the same applications

▪C5011:

- Cartridge: Same as C2011 but with 2X more tubing for oxygenation and larger pump tubing for higher flow rates.
- Recommended for: Monoclonal antibody production.
- Protein Production: Can produce 10-100 mgs of monoclonal antibody every two days.
- Medium Consumption: Up to 1 liter of medium every day.

▪C2018:

- Protein Range: 1 kDa - 10 kDa, greater than 80 kDa
- Recommended for: Recombinant protein production and exsome production.
- MWCO: Usefully retains proteins greater than 100 kDa.
- Capacity: Can support up to 5X1010 cells.
- Protein Production: 5-25 mgs of recombinant protein every day.

▪C2008:

- Protein Range: 10 kDa - 100 kDa
- Recommended for: Adherent and suspension cell types.
- MWCO: Low molecular weight cut-off.
- Secreted Proteins: Secreted proteins in the range of 20 kD and

higher will be retained in the small volume of the ECS (20mls).
- o Capacity: Can support up to 109 cells.
- o Medium Consumption: Up to 1 liter of medium per day.

▪C2003:

- o Protein Range: 10 kDa - 80 kDa
- o Recommended for: Adherent and suspension cell types.
- o MWCO: Low molecular weight cut-off.
- o Secreted Proteins: Secreted proteins in the range of 20 kDa and higher will be retained in the small volume of the ECS.
- o Capacity: Can support up to $5X10^{10}$ cells.
- o Medium Consumption: Up to 4 liters of medium per day.

These cartridges are designed to cater to different cell culture needs, protein production requirements, and medium consumption rates. The choice of the appropriate cartridge depends on the specific application and desired outcomes. Feel free to contact our customer support team at **info@fibercellsystems.com** and **+1 301 471 1269** for further assistance or to discuss which cartridge best suits your research or biopharmaceutical processes.

2.5 Basic Laboratory Procedures for Hollow Fiber Bioreactors (for all applications)

Technique

- Correct sterile technique will ensure a long and productive life for your hollow fiber module. Shortcuts, suspect medium, and poor sterile technique may result in contamination.
- Use a needle to draw liquids into syringes. Droplets of medium at the syringe/side port junction invite contamination.
- Disconnect the needle from the syringe after loading and attach syringe directly to the ECS port.
- Perform all operations in the laminar flow hood. Keep the hood clean. Avoid rapid movements and working directly over the samples.
- Always wear a lab coat and gloves or sterile sleeves. Always observe good sterile technique.
- Replace syringes with fresh ones whenever the cartridge has come out of the laminar flow hood.

Cells

- FiberCell Systems hydrophilic polysulfone fibers are appropriate for the culture of both adherent and suspension cell lines.

- Cells should be at least 90% viable. Minimize the time between cell harvesting and inoculation into the cartridge.

Media

- Standard Mediums: Use the same medium used to grow the cells of choice in flask culture, initially. The use of a high glucose (4.5 g/L) medium is strongly recommended therefore the use of medium like low glucose RPMI is to be avoided if possible.

- CDM HD: If you wish to use protein free CDM HD serum replacement, utilize standard mediums during the growth phase and once the after the cells have reached high density inside the hollow fiber module you can switch directly to your basal medium with 10% CDM HD. High cell density is defined as a glucose uptake rate of greater than 1 gram per day in the C2011 cartridge. Follow the CDM HD usage instructions found in section 6 of this manual. For many cell lines including hybridoma, CHO, BHK, and 293 cell lines, excellent results can be obtained using protein-free CDM HD from FiberCell Systems. No adaptation is required. Please refer to our website, www.fibercellsystems.com or contact FiberCell Systems, **info@fibercellsystems.com,** for more information. It is much easier to adapt the cells to a different medium after the cells have reached a high density inside the cartridge than to do so in a flask or spinner culture.

- Warm media and reagents in a 37ºC water bath. Wipe bottles down with alcohol before putting in the laminar flow hood. The reduced pressure inside a cold bottle of medium will draw in air upon opening.

- Pipette, NEVER POUR, media and reagents.

3.0 Quick Start Guide

Preparation/Getting Ready for setting up the system for all applications: (please refer to the video “setting up the cartridge” found here: **https://www.youtube.com/watch?v=-bmxOUk3ElE&t=184s**

This section covers all the set up and handling of the cartridge.

3.1 Reservoir Bottle And Cap Assembly Sterilization

Each cartridge comes with two short pieces of tubing in the outer bag. Being careful not to tear the inner bag, remove the two pieces of tubing. These will be connected to the cap before autoclaving.

Figure 13: Aluminum foil protects the exposed parts of the reservoir cap. Once placed in autoclave bag the reservoir is ready to be autoclaved. Place the reservoir cap tubing on the hose barbs of the cap prior to autoclaving.

1. Hold the reservoir cap up to a sterile 250 mls Nalgene bottle. The stainless steel tubes should reach within a ½" of the bottom of the bottle in order to maintain a constant media flow through the cartridge. If the tubes are too high, wet the tubing around the cap with DI water and they will slide up and down easily. Adjust to correct height. If using the 45 mm cap, the tubing is not adjustable but designed to reach the bottom of a 500 mls Gibco bottle.

2. Attach the two pieces of reservoir cap tubing supplied with each cartridge to the hose barb fittings on the reservoir cap and cover with aluminum foil. Cover lower end of tubing on reservoir cap and luers with aluminum foil and secure with autoclave tape.

3. Place the reservoir cap assembly into an autoclave bag.

4. Autoclave the reservoir cap at 120–130°C for 45-60 min.

5. If your autoclave does not have a dry cycle, place the autoclave bag into the laminar flow hood immediately after removal from the autoclave. The wet paper side of an autoclave bag is not a barrier to contamination.

NOTE: There is no directional orientation for the stainless steel tubes. The inlet and outlet tubing may be connected to either luer fitting on the reservoir cap.

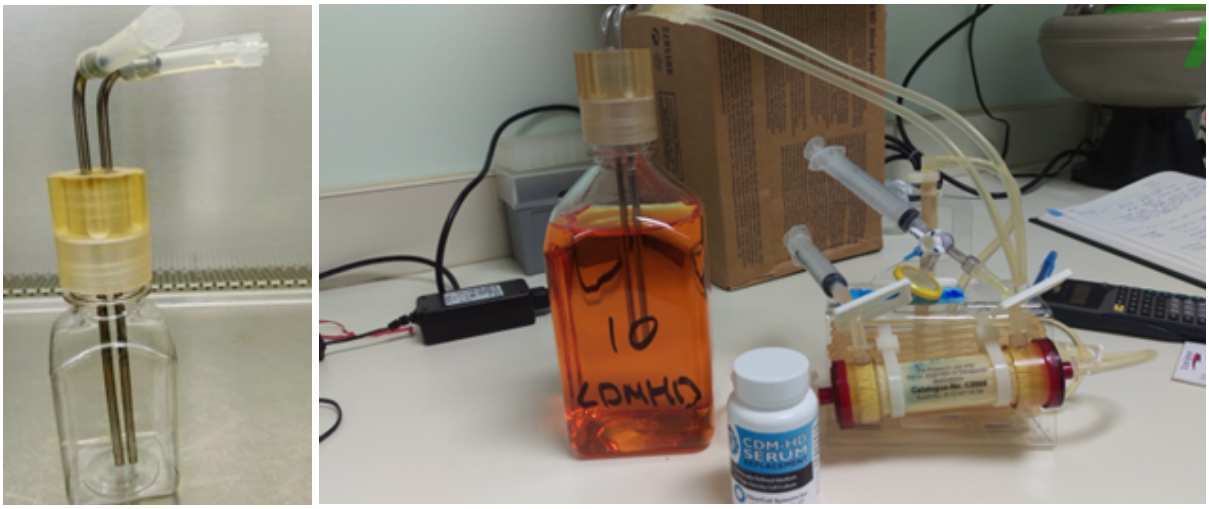

Figure 14: FiberCell Systems' Reservoir Cap.

3.2 Pre-Culture

Please watch "Setting up a Fibercell Systems Hollow Fiber Bioreactor" here: https://www.youtube.com/watch?v=-bmxOUk3EIE

Pre-Culture Stage for All Cell Culture applications.

First Step: Set up the cartridges with PBS
Second Step: Change to basal medium
Third Step: Change to complete medium (with FBS)
Fourth Step: Change to fresh complete medium

The purpose of this pre-culture is to:

- remove the wetting agent from the fibers
- equilibrate the system with growth medium and serum proteins
- verify that the system is leak free
- perform a sterility check

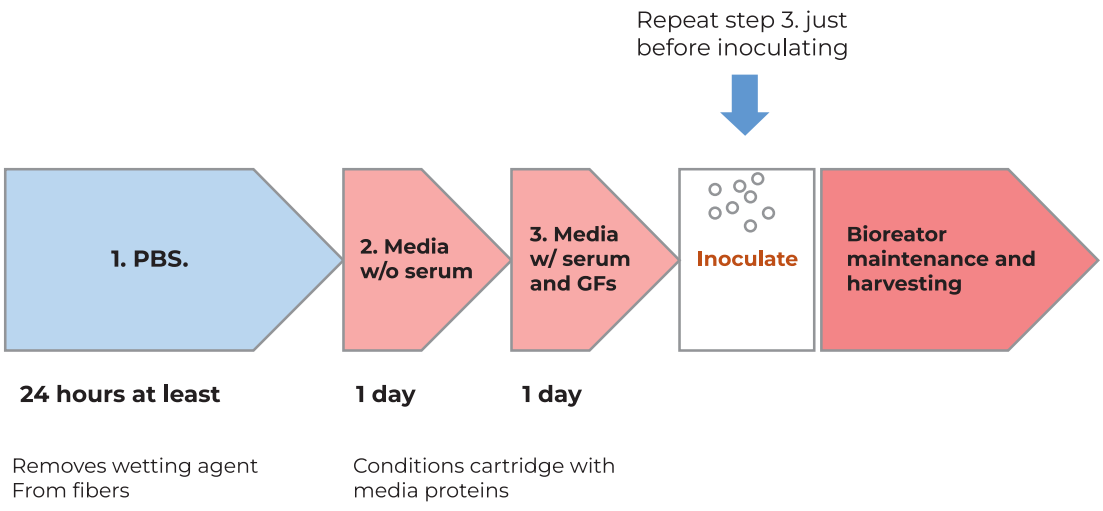

Figure 15: Block diagram of steps involved in setting up and seeding a cartridge.

Materials/Tools Required

Cells will not be inoculated until several days later and are not required at this time. . Please have the following materials on hand in the hood prior to starting:

- Sterile 500 mls bottle of PBS
- FiberCell Systems culture module
- FiberCell Systems reservoir cap, autoclaved, with tubing attached
- 60cc and/or 20 cc sterile syringes (luer-lock)
- Alcohol pads
- Spray bottle containing 70% ethanol
- Large 14-18 gauge needles – for safety blunt end needles are recommended
- 50 mls sterile conical centrifuge tube filled with 50 mls PBS for filling the ECS
- 25 mls or 50 mls pipettes

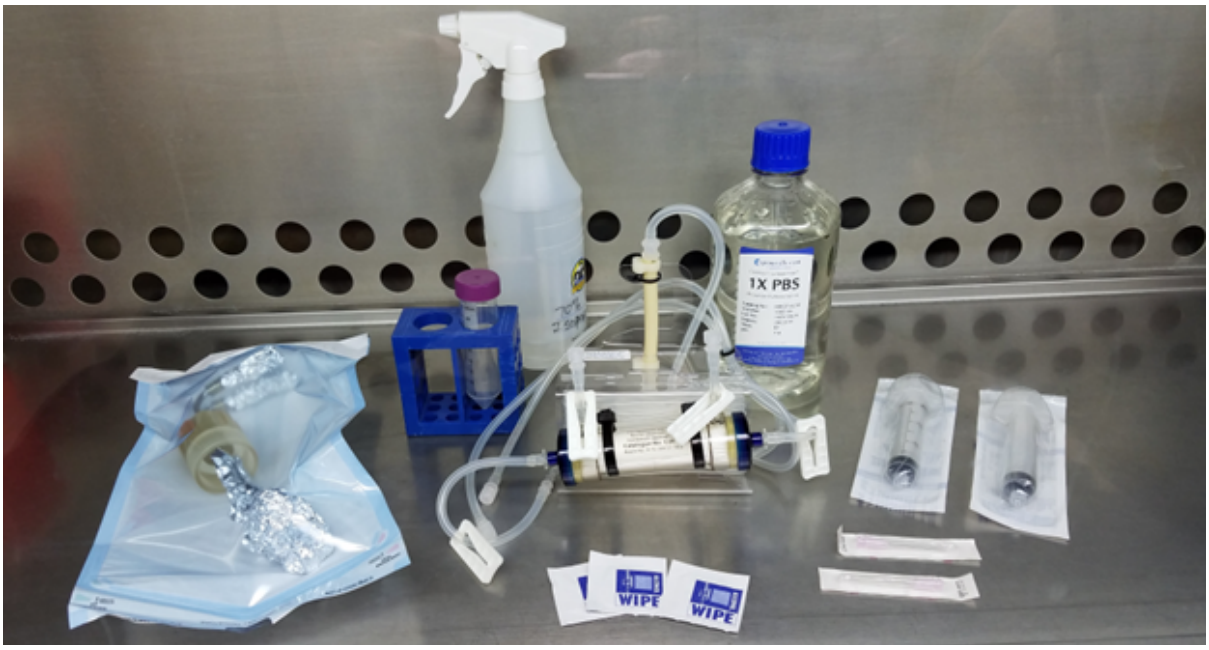

Figure 16: Cartridge and materials for setting up the cartridge.

3.2.1 Attach, prime and fill the ECS with PBS

Attach and Prime

1. Pipette 50 mls out of the 500 mls bottle of PBS and place into the 50 mls conical tube, carefully take the reservoir cap out of the autoclave

pouch, remove the tin foil on the ends of the tubing and replace PBS bottle cap with the reservoir cap. It is easiest to rotate the bottle under the cap.
2. Attach the tubing from the cartridge to the luer connections on the reservoir cap. Give each tube from the cartridge ½ backspin so tubing doesn't kink.
3. Check that the left and right end port slide clamps are in the OPEN position and both left and right ECS ports are closed.
4. Perfuse medium through the flow path circuit by pumping the pump tubing with your fingers until the circuit is filled and no bubbles come from the stainless steel tubing inside the reservoir bottle.
5. Tilt the cartridge with the right side up to purge any air bubbles that may have collected in the fibers or at the ends of the bioreactor.

Fill the ECS with PBS

1. Close the left and right end port slide clamps on the cartridge to isolate the bioreactor from the flow path.
2. Attach a sterile syringe to the right ECS side port.
3. Fill a second syringe with 20 mls of PBS using the large gauge needle, remove needle and connect syringe to the left ECS side port.
4. Open the left and right ECS slide clamps.
5. Inject the PBS into the ECS displacing the air into the other syringe, tilting the right side of the cartridge up. If the ECS is not completely filled with medium, refill syringe and repeat, dislodging all air bubbles.
6. Close the ECS side port clamps, remove any air from the syringes and use the syringes as caps. Use fresh syringes for subsequent manipulations.
7. Open the left and right end port slide clamps.
8. Place the cartridge onto the Duet and run PBS through the system at a flow setting of 15-20 for a minimum of 24 hours. At this point the cartridge may be run with the PBS for several weeks if necessary.

3.2.2 First Media Change

1. This first liquid change will be using classical media/serum free media. Replace the PBS in the reservoir bottle with a 125 mls of classical media.
2. Close the left and right end port slide clamps. Close the left and right ECS side port slide clamps.
3. Change the medium in the ECS by filling a 20 mls syringe with the new medium and attaching it to the left ECS side port. Place an empty syringe onto the right ECS side port.
4. Open the ECS port slide clamps.
5. Tilt the cartridge up on the right side and exchange the medium in the ECS by slowly pushing the new medium in from the left syringe and floating the old medium out the right ECS port.
6. Remove the PBS from the right syringe and reattach it to the ECS port.
7. Close the clamps on the left and right ECS ports. Remember to open the left and right end port slide clamps!

8. Let the media circulate for a minimum of 24 hours, placing the system back in the incubator. Flow setting should be 20-25 on the controller.

3.2.3 Second Media Change

1. Follow steps in first media change.
2. If using a basal medium, change out the 125 mls DMEM for 125 mls DMEM plus 10% fetal bovine serum and any other additives such as antibiotics. Allow this to circulate for 24 hours. Exchange the medium in the ECS as well.

3.2.4 Final Media Change

Put on a fresh change of DMEM plus 10% fetal bovine serum, 125 mls or Larger volumes have been used as a convenience to this point. However during the initial seeding period it is important that the volume be no more than 125 mls. The volume of media in the reservoir bottle needs to remain proportional to the number of cells in the cartridge.

You are now ready to inoculate with cells.

Important to remember:

- Use the alcohol swabs to clean up any medium on the luer fittings or cartridge.

- Always have either a syringe or cap on the ECS side ports, or the slide clamps closed to prevent excess medium from collecting on the fittings or leaking.

- In the event of ECS drainage, (ECS fills with air overnight during pre-culture) raise the level of the reservoir bottle so that the level of the medium in the reservoir is higher than the ECS. This will generate sufficient hydrostatic pressure to keep the ECS filled with medium. Also, ensure that the ECS port slide clamps are closed and the luer caps or syringes are tightly fitted.

- After this 24-hour flush, there will be two more changes of medium in the system. One of basal medium without serum or growth factors (or serum free medium) and then a second change of complete medium containing serum, antibiotics, and any other additives.

- Finally, perform a fresh change of medium for cell inoculation and initial culture. When the cells are established, i.e., consuming one gram of glucose a day or more, adaption to serum free media or CDM HD can be performed. CDM HD requires little or no adaptation. Simply replace the fetal bovine serum in the DMEM with 10% CDM HD.

CDM HD instructions may be found at:**http://www.fibercellsystems.com/products/cdm-hd-chemically-defined-high-density-serum-replacement/**

3.3 Cell Inoculation

Refer to video: **"Inoculating Cells into a FiberCell Systems Hollow Fiber Bioreactor" https://www.youtube.com/watch?v=x_B7PtRixew**

Please refer to the FiberCell Systems website, www.fibercellsystems.com for access to training videos.

Make sure that the cells are at least 90% viable. Minimize the amount of time between cell harvesting and inoculation. Re-suspend the cells in the same conditioned medium that they have been growing in as this will contain useful growth factors that should not be discarded. Recommended cell numbers are given here:

- Hybridomas and suspension cells: a minimum of 108 total cells.
- Adherent Cells such as CHO and HEK 293: use the equivalent of 50% confluence of the fiber surface area. This will be 6-8 T- 175 flasks or the equivalent.

3.3.1 20 kDa MWCO Cartridge Cell Inoculation

1. will remain in the cartridge while the excess medium will go into the reservoir bottle.
2. Close the ECS slide clamp and repeat with the opposite syringe, remembering to close ECS ports after expelling cell suspension and opening the left end port slide clamp.
3. Tighten the reservoir cap.
4. Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.
5. Leave these syringes on to help guard against contamination.
6. Place the cartridge onto the pump and set pump speed to 20-25.
7. Flow rate for C2025D should never exceed a pump setting of 10.
8. Close one of the ECS side port slide clamps. It does not matter which you start with. Open the right end port slide clamp.
9. Crack the reservoir cap by 1/4 turn. Gently push the suspension in the other syringe through the fibers and into the reservoir bottle. The cells will remain in the cartridge while the excess medium will go into the reservoir bottle.
10. Close the ECS slide clamp and repeat with the opposite syringe, remembering to close ECS ports after expelling cell suspension and opening the left end port slide clamp.
11. Tighten the reservoir cap.
12. Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.

13. Leave these syringes on to help guard against contamination.
14. Place the cartridge onto the pump and set pump speed to 20-25.

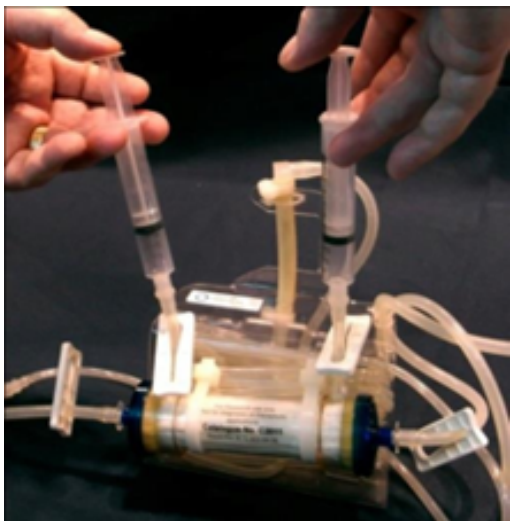

Figure 17: Cell Inoculation Using Syringes.

3.3.2 5 kDa MWCO Cartridge Cell Inoculation (C2008, C2003)

A different protocol is required for the 5 kDa MWCO cartridges as it is not possible to achieve bulk flow of liquid across the lower MWCO fibers.

1. Close end port slide clamps.
2. Attach a fresh 20 mls syringe to the left ECS side port. Attach an empty 20 mls syringe to right ECS side port with plunger pulled back so it is full of air.
3. Open ECS side port slide clamps.
4. Tilt cartridge and withdraw medium from ECS into the empty syringe, pulling air from the other syringe (this keeps you from just pulling air from the hood into the ECS).
5. Note volume removed, should be around 10-12 mls Expel remaining air from right ECS port syringe and re-attach.
6. Resuspend cells to be inoculated in the volume removed plus 2 mls (12-14 mls).
7. Fill syringe with cell inoculum.
8. Depress plunger and push in cells, swish back and forth, 1 ml remains in each syringe.
9. Crack open reservoir cap ¼ turn. Close one ECS side port clamp push in one ml of excess medium and repeat with other ECSport. It should be possible to easily get 2 mls through the fiber. Gentle, steady pressure.
10. Make sure both the ECS side port clamps are closed and end port slide clamps are open. Tighten the reservoir cap.
11. Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.
12. Place the cartridge onto the pump and set pump speed to 20-25.

3.4 Daily Maintenance

The below chart shows the steps to take for the first 7-10 days of culture Once the cells have reached high density in the bioreactor (defined as the consumption of 1 gram of glucose per day or more) all the needs to be done is the measurement of glucose, changing the medium, and harvesting product (detailed in section 3.5). Measurement of glucose is critical (see section 3.4.1) You may post the Daily Maintenance Schedule (below) in your workspace to keep track of maintenance requirements for your FiberCell System.

Protocol Summary – Routine Daily Maintenance

DAY	CHECK GLUCOSE	CALCULATE RATE	RESERVOIR VOL.	ACTION (S)
0	YES	N/A	100	• INOCULATE CELLS • ATTACHMENT • FLOW RATE AT 2 5 on control knob
1	YES	YES	100	DOUBLE MEDIUM IN RB IF GLUCOSE IS 50% DEPLETED
2 - 5	YES DAILY	YES DAILY	200-500	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED
5 - 7	YES DAILY	YES DAILY	500 - 1000	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • HARVEST SECRETED PROTEIN FROM ECS
5 - 7	YES DAILY	YES DAILY	500 - 1000	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • HARVEST SECRETED PROTEIN FROM ECS
7 - 10	YES DAILY	YES DAILY	1000 (2L to 4L for large cartridges)	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • HARVEST Mab FROM ECS EVERY TWO DAYS
10 +	YES DAILY	YES DAILY	1000 (2L to 4L for large cartridges)	• REPLACE MEDIUM BOTTLE WHEN GLUCOSE IS 50% DEPLETED HARVEST MAB FROM ECS E VERY TWO DAYS • REDUCE SERUM IF DESIRED • MONITOR CELL MASS IN ECS AND FLUSH AS REQUIRED

3.4.1 Glucose Monitoring (Measurement of Glucose and the Glucose Uptake Rate)

Please refer to the video "Measuring Glucose from a FiberCell Systems Hollow Fiber Bioreactor" https://www.youtube.com/watch?v=dti3dqJKei8 Measurement of glucose and calculating the glucose uptake rate is critically important in the maintenance of a hollow fiber bioreactor. It is difficult to visualize the cells, though small samples can be pulled out and placed into a flask for visualization and as a check for proliferative capability. However, when taking a sample, the non-viable cells will be preferentially collected as they are not adherent to the fiber. This sample will not reflect the overall status of the cartridge. Measurement of the glucose can tell us two things:

1. The total amount of glucose in the medium tells us when it is time to change the medium. We want to maintain culture conditions in a homeostatic manner, which means changing the medium before the pH changes, and the color of the medium visibly changes. The medium should be changed when the measured glucose is 50% or less of the original concentration. For high glucose DMEM (Dulbecco's Modified Eagle Medium) which has a starting glucose of 4.5 grams per liter, you will want to change the medium when it is 2.25 grams per liter or less. This guideline applies to most media. If you are not sure of the starting glucose check with the manufacturer of the medium.

2. The glucose uptake rate, i.e. the amount of glucose consumed by the cartridge in 24 hours indicates how many cells are in the cartridge, and how healthy they are. If the rate has plateaued then the cells are in a steady state, if the rate is increasing then the cells are proliferating. If the glucose uptake rate drops then cell number is reduced, cells are dying or something else is going on inside the bioreactor. We know empirically that a glucose uptake rate of 1 gram per day corresponds to approximately 1x109 cells. The ideal glucose uptake rate for the C2011 and C2008 cartridges is between 1-1.5 grams per day. For the C2018 and C2003 cartridges is it between 2-6 grams per day. It is important to keep the glucose uptake rate below 2 grams per day in the C2011 and C2008 cartridges to prevent anaerobic metabolism. The glucose uptake rate indicates which type of harvest should be performed, low glucose rate or high glucose rate, and the approximate number of cells you will want to remove.

Glucose uptake rate is calculated by taking the starting glucose measurement and subtracting from it. the glucose measurement 24 hours later. Since the rate is dependent upon the size of the reservoir bottle you multiply the drop in glucose in grams by the volume of media in the reservoir bottle. For example, if there are 250 mls in the reservoir bottle, multiply by .25 or 25%.

(Glucose1-Glucose2) X (reservoir bottle in liters). 250 mls is .25 L. If more than one day has elapsed, divide this by the number of days.

For example:

- Starting glucose 4.5 grams per liter
- Measure glucose 24 hours later 2.7 grams per liter
- Reservoir bottle 200 mls

  4.5-2.7=1.8 grams of glucose

  1.8X.25 (reservoir bottle is .25 liters), .45 grams of glucose consumed

  Glucose uptake rate is 450 mgs per day.

We recommend the GlucCell, a glucose meter designed specifically for cell culture media.. Newer diabetic glucose meters are not compatible with cell culture media.

https://chemglass.com/gluccell-glucose-monitoring-system

When the glucose consumption rate is 1.0 gram per day or higher, harvesting may begin. At this glucose rate the cells are at high density and CDM HD may be substituted for serum. At a glucose rate of less than 1 gram per day only a low glucose rate harvest should be performed. Between 1-2 grams per day a low glucose rate harvest should be performed, followed by a high glucose rate harvest. If you have any questions please feel free to contact FiberCell Systems at **info@fibercellsystems.com** and **+1 301 471 1269.**

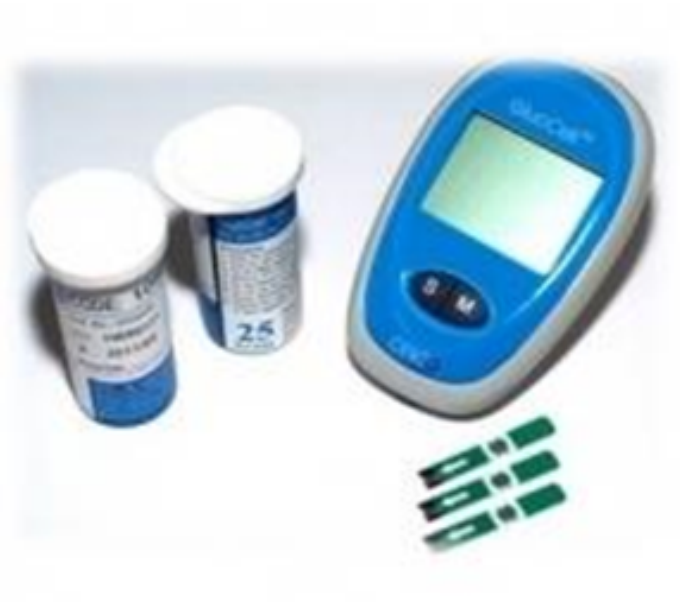

Figure 18: GlucCell classical media glucose monitor or chemical analyzer for measuring glucose. Diabetic meters and test strips will not work with classical cell culture media.

3.5 Harvesting

Please refer to the videos "Low glucose Rate Harvesting" and "High glucose Rate harvesting"
https://www.youtube.com/watch?v=n3NxIG-7AOI

https://www.youtube.com/watch?v=elbvGep8Js4&t=5s

Harvesting from a hybridoma culture should be performed every other day, for CHO or 293 cell lines producing a recombinant protein and other secreted products such as exosomes, harvesting should be performed every day, if possible. Harvesting from the cartridge is intended to accomplish two things. 1) Harvest the secreted product as concentrated as possible. 2) Control the cell mass and keep it from getting too high. This prevents anaerobic metabolism. Removal of dead cells is also important. If there are too many cells in the cartridge then it is possible to exceed the capacity of the system to deliver oxygen. This can drive the cells into anaerobic metabolism. Once the cells are in anaerobic metabolism it can be difficult to get them to recover. The low glucose rate harvest will provide the highest concentration of product but will not remove many cells. The high glucose rate harvest will remove cells and keep the pores of the fiber open but will dilute the product.

The low glucose rate harvest should always be performed first, followed by the high glucose rate harvest if the glucose rate is above 1.5 grams per day, or at least once a week. The type of harvest performed is determined by the glucose uptake rate.

3.5.1 Low Glucose Rate Harvest

If the glucose rate is below 1000 mgs per day, harvesting should be done so that only a few cells are removed.

Equipment and Materials

FiberCell Systems cartridge
20 cc sterile syringes (luer-lock)
Alcohol pads
Spray bottle containing 70% ethanol or isopropyl alcohol
Cell culture media of choice

Procedure

1. Close the left and right end port slide clamps. Make sure the ECS port slide clamps are closed.

2. Fill a 20 mls syringe with the fresh complete medium. Remove the-syringe off the left ECS port and replace with the syringe containing fresh medium. Place a new, empty syringe on the right ECS port. Open both the left and right ECS port slide clamps.

3. Tilt the cartridge up on the right side and exchange the medium in the ECS by slowly pushing the new medium in from the left syringe and floating the supernatant out the right ECS port syringe. (Please refer to picture in pre-culture section). This will represent your low glucose rate harvest. This type of harvest is the most concentrated.

4. Close both the ECS slide port clamps. Remove the syringe containing the harvest and replace with fresh sterile syringe.

5. Before putting back into the incubator, be sure that both the inlet and outlet end port slide clamps are open. It is a good idea to pump the media with your fingers to be sure the media is circulating.

3.5.2 High Glucose Rate Harvest

Use this procedure when the glucose rate is 1000 mgs per day or above which indicates that the cell mass needs to be controlled and cell numbers reduced. The cell pellet may be 1-4 mls of packed cells.

Equipment and Materials

FiberCell Systems cartridge
20 mls sterile syringes (luer-lock)
Alcohol pads
Spray bottle containing 70% ethanol or isopropyl alcohol

Procedure for 20 kDa MWCO Cartridge High Glucose Rate Harvest (C2025D, C2011, C2018)

1. Close the left end port slide clamp (right end port remains open).
2. Attach two fresh 20 mls syringes to the ECS side ports.
3. Crack the reservoir bottle cap about 1/4 turn.
4. Open the right ECS port slide clamp.
5. Pull 10 mls gently into the right syringe (you are pulling medium out of the reservoir bottle, through the fibers, into the syringe.
6. Close the right ECS port slide clamp.
7. Open the left ECS port slide clamp.
8. Pull 10 mls of medium into the syringe.
9. Close the right end port slide clamp.
10. Making sure both the left and right end port slide clamps are closed, open both ECS port slide clamps.
11. Swish the medium between the two syringes, 2-3 times, gently. The higher the glucose uptake rate, the more swishes you should use.
12. Push all the media into one of the syringes, it doesn't matter which one.
13. Close the ECS side port slide clamps. Remove the syringe containing the medium and empty it into a 50 mls conical.
14. Replace the syringe.
15. Open the left and right end port slide clamps. Tighten the reservoir cap.

Procedure for 5 kDa MWCO Cartridge High Glucose Rate Harvest (C2008, C2018)

1. Close left end port slide clamp, leave right end port slide clamp open.
2. Attach fresh syringes on ECS side ports, one filled with 10 mls of medium (right), and the other empty with the plunger all the way down (left).
3. Open the left ECS side port slide clamp.
4. Open the reservoir bottle cap 1/4 turn.
5. Pull 1 ml of medium into the left syringe.
6. Close the left ECS side port clamp; open the right ECS side port clamp.
7. Pull 1 ml medium into the right syringe.
8. Close right end port slide clamp, open left ECS side port slide clamp.
9. Swish medium back and forth between the syringes 3-4 times. Push all the medium into one syringe. This is your harvest.
10. Close left and right ECS side port slide clamps.
11. Open left and right end port slide clamps. Tighten the reservoir cap.
12. Place harvest into a 50 mls conical tube and spin down.
13. Re-attach syringes to the ECS side ports. These will serve as end caps.

3.5.3 Harvesting using 3-way stopcock and sterile filter.

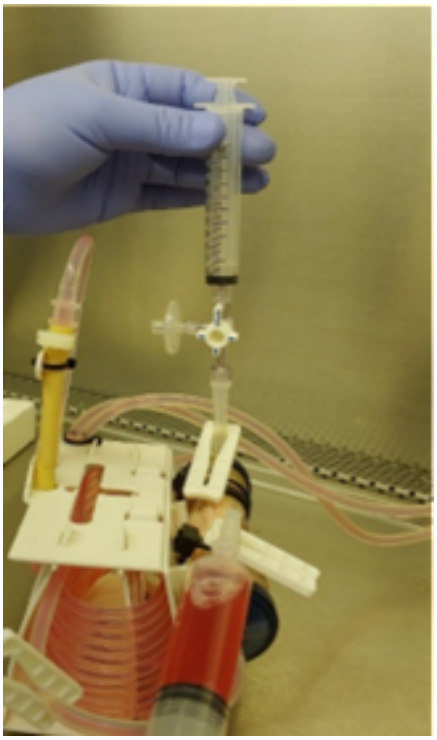

Figure 19: The Use of a 3-Way Stopcock and a Sterile Filter in Harvesting.

It is important to remove the proper number of cells during the harvest procedure to maintain the glucose uptake rate at the correct level. Over the years we have developed the following method in our own laboratory. It is designed to result in the most concentrated harvests while removing the correct numbers of cells for most applications. It works especially well for hybridomas. 293 cells may also require some additional cell harvests to control the cell mass and glucose uptake rate. This protocol uses a 3-way stop cock and sterile filter on the right side port to ensure that the cartridge remains sterile during this procedure.

1) Close left end port slide clamp.
2) Place 3-way stop cock and sterile filter on the right side port (as shown in the photo)
3) Attach a fresh 20 mls syringe to the left side port
4) Close both the left and the right end port slide clamps
5) Open both the left and the right side port slide clamps
6) Turn the 3-way stop cock so that flow goes through the filter, and into the side port.
7) Raise the right end of the cartridge and drain the ECS completely into

the empty syringe on the left side port. This is your harvest.
8) Close the left end port slide clamp, remove the syringe, and collect the harvest into a 50 mls conical tube.
9) Replace the empty syringe onto the left side port.
10) Open left side port
11) Close left side port
12) Open left end port slide clamp and open the reservoir cap by ¼ turn
13) Fill the ECS with medium from the reservoir bottle by with drawing on the left syringe and pulling until the ECS is filled. Do not pull medium into the syringe.
14) Close the right end port slide clamp
15) Open the right side port slide clamp
16) Drain the ECS into the syringe a second time.
17) Close the left side port slide clamp
18) Collect harvest into a 50 mls conical tube
19) Replace syringe on the left side port
20) Close the left side port slide clamp
21) Open right end port slide clamp
22) Fill the ECS by pulling medium from the reservoir bottle, though the fibers, and into the ECS. Fill the ECS but do not pull any medium into the syringe.
23) Close left and right side port slide clamps
24) Tighten the reservoir bottle cap.
25) Open the left end port slide clamp (Don't forget to open the left end port slide clamp!)
26) Return cartridge to the pump in the incubator.

This harvesting procedure will provide the most concentrated product, and in most cases will remove the proper number of cells from the cartridge, while at the same time ensuring that the cartridge will remain sterile. Do not place the 3-way stop cock and filter on the cartridge until after the cells have been seeded as they are incompatible with the cell inoculation protocol. This method will work only with the 20kDa MWCO fiber as the 5kDa MWCO fiber does not allow for bulk flow of liquid through the fiber.

Harvesting Tips

- Do not start harvesting until you have a glucose uptake rate of 1 gram per day and have consumed the first liter of medium.

- It can take 3-5 flushes of the ECS to remove most of the serum proteins after switching to CDM HD.

- Gauge the number of cells to remove, and the type of harvest(s) to perform (low glucose rate, high glucose rate or both) based upon the glucose uptake rate. With the C2011 do not allow the glucose uptake rate to get above 2 grams per day, with the C5011 do not allow the glucose uptake rate to get above 4 grams per day.

- Harvest every other day to allow antibody to concentrate in the ECS.

- One of the most common mistakes people make is to not harvest and remove enough cells when the glucose uptake rate is too high. It is normal to have a cell pellet harvest of 2-4 mls.

Purification Tips

- Be sure to spin down the harvests immediately after collection to remove cells from the supernatant. This will prevent antibody degradation and contamination with host cell proteins.

- When using CDM HD you may be able to simplify purification protocols as the antibody of interest will be in the range of .5 mgs/mls to 5 mgs/mls and be the primary protein present.

- When using CDM HD avoid phosphate buffers. CDM HD contains a significant amount of free iron (there is no transferrin in CDM HD) that can interact with phosphate buffers. If you are planning on using Protein A or Protein G for antibody purification, please check the CDM HD usage instructions for alternate buffers at

https://www.fibercellsystems.com/wp-content/uploads/2018/12/4-5-CDM-HDUsageInstructions-1.pdf.

3.6. Flow Rate

Generally it is not possible to have too high of a flow rate in a hollow fiber bioreactor in the medium (C2011, C2008) and large (C2003 and C2018) cartridges from FiberCell Systems. There are 4,000 to 6,000 fibers in these cartridges and the total flow rate is divided by this number of fibers. The flow rate per fiber then is very small. The resistance to flow is also low so no significant backpressure is generated in these systems. For most applications it is best to simply set the control knob to a flow rate of 25. If the glucose uptake rate is especially high set the flow rate to maximum.

Flow rates for the smaller C2025D cartridges should never be above a setting of 10 on the control box. The small size of the cartridge CAN result in damaging pressures if the flow rate is too high. The C2025 PVDF cartridge is primarily intended for use with endothelial cells seeded on the insides of the fibers and flow rates are based upon the shear stress the cells should be subjected to. Refer to the “Endothelial Cell Culture Instructions on the FiberCell Systems website.

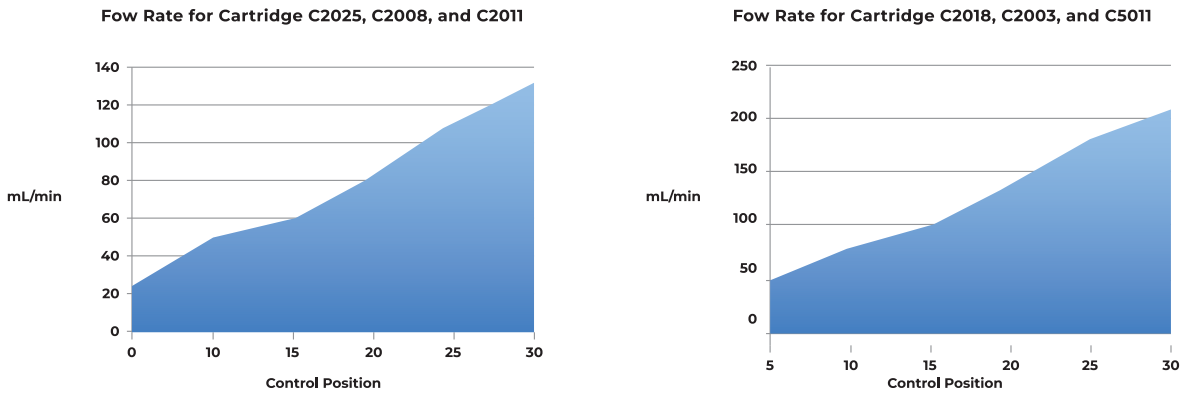

Figure 20: Duet flow rates vs. control knob position.

4.0 Protocol Specific applications:

4.1 Monoclonal Antibodies

The primary difference in protocols between recombinant protein production and monoclonal antibody production is that hybridoma cultures should be harvested every two days. The molecular weight cut-off should be the 20 kDa MWCO, and the specified cartridge is the C5011. This cartridge allows for TGF-beta to diffuse away while retaining the cells and secreted antibody in the ECS. The higher oxygenation capacity of the C5011 ensures your cells will not go into anaerobic metabolism.

Please refer to the white papers, application notes and webinars on the FiberCell Website at https://www.fibercellsystems.com/technical-resources/technical-documents/.

Hybridoma Tips:

Cholesterol: Some clones, i.e., NSO, can be cholesterol dependent. In this case artificial cholesterol in serum free mediums is bound up with cyclo-dextrin which will bind to the silicone tubing. With cholesterol dependent cell lines, it is recommended to culture using DMEM/10% CDM HD with .5-1% FBS in the circulating medium only. The cholesterol and other nutrients can cross the fiber, but bovine IgG and other large components cannot cross the fibers.

- Ensure that you are using medium based upon the FiberCell Systems guidelines. Medium should be DMEM or other high glucose mediums. Nothing less than 3.5 g/L should be used.

- Ensure that there are no additives such as insulin, transferrin, or other supplements not specifically recommended. These are not required.

- Use a good grade of serum, bad lots of serum can affect growth. “Good” serum is triple 100nm filtered, full 9CFR and mycoplasma tested and source traceable.

- Ensure that the end port slide clamps are open, the side port slide clamps are closed.

  At this point it is a good idea to pull out a small sample of cells and check for viability using Trypan Blue. Another good viability check is to place a sample of cells from the ECS into a T25 flask with serum containing medium. High viability coupled with no or low glucose consumption is an indication that the cells are in lag phase. This means the volume of the medium is too large for the number of cells and the conditioning factors, specifically IL6 are too dilute to support cell growth. What to do?

- Reduce the volume of the medium in the reservoir bottle to 75 mls or less.

- Seed more cells, an additional 1 x 108 at the very least.

- Increase serum to 20%.

- Reservoir cap and reservoir bottle size: FiberCell Systems offers three different sizes of reservoir caps: 33 mm to fit onto glass media bottles with the black plastic cap, 38 mm to fit onto most standard Nalgene media bottles, and 45 mm to fit onto the wide mouth Gibco media bottles. The most common is the 38 mm reservoir cap. The length of the stainless-steel tubing can be adjusted after running the cap under some water. When you first get the reservoir cap adjust the length so that the tubes reach the bottom of a Nalgene 250 mls media bottle. This will allow you to use a 250 mls bottle to initiate the culture with 125 mls of medium and permit the next step of 250 mls It will also allow you to use 500 mls in a 1-liter bottle. The 45mm cap reaches the bottom of a 500 mls Gibco bottle. The size of the reservoir bottle is most important for hybridoma culture.

- Hybridoma culture in the hollow fiber bioreactor presents a balancing act between two cytokines 1) TGF-Beta which is inhibitory and 2) IL6 which is stimulatory. Different hybridoma clones have differing sensitivities to these two cytokines. In some cases when changing the medium and increasing the volume in the reservoir bottle during culture initiation it is useful to add fresh medium on top of the conditioned medium rather than removing and replacing 100% of the conditioned medium in the reservoir bottle. For example, after the first 125 mls of medium has its glucose depleted by 50% add 125 mls of fresh medium to the existing 125 mls for a final volume of 250 mls.

4.2 Recombinant Proteins

Difficult-to-express proteins are generally considered to be recombinant proteins that are expressed at low titers in mammalian systems, but can 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 systems versus other hosts such as bacteria, insect cells and yeast. Mammalian cells are the preferred platform to produce 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.

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

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.

Expression of proteins and especially difficult-to-express proteins in mammalian expression systems is efficient and cost effective in a HFBR. There are many advantages to 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

A 3-D hollow fiber bioreactor can be the most effective way to simulate various in vivo biological processes. For other specific application please refer to the published literature or feel free to contact FiberCell technical support.

4.3 Extracellular Vesicles and Mesenchymal Stem Cells

4.3.1 Overview: Mesenchymal Stem Cells (MSCs)

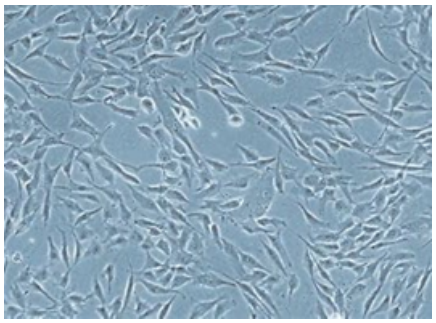

Figure 21: Mesenchymal stem cells in 2-D flask culture.

Mesenchymal stem cells (MSC) are multipotent adult stem cells that are present in multiple tissues, including umbilical cord, bone marrow and adipose tissue. Mesenchymal stem cells can self- renew and can also differentiate into bone, cartilage, muscle, adipocytes, and connective tissue. Extracellular vesicles from MSC have demonstrated strong regenerative capacity that may treat a variety of medical conditions. The International Society for Cell and Gene Therapy recommended minimal criteria to define MSCs as the following: (1) cell adhesion to a plastic surface, (2) specific positive and negative surface markers and (3) in vitro tri-lineage differentiation capability.

The unequivocal identification of MSCs in vivo has been hindered by their extremely low frequency in tissues and the lack of a distinct MSC-specific immunophenotype to enable their identification and isolation. The translation of exosome therapies to clinical applications has been hindered by the difficulty in producing clinically relevant quantities of extracellular vesicles from MSC and other cell types. A hollow fiber bioreactor represents a readily available methodology to produce large quantities of extracellular vesicles from MSC and other cells in a cGMP compatible system.

3-D Artificial Capillary Hollow Fiber Bioreactors vs. T-Flasks

3-D hollow fiber artificial capillary bioreactors present a more in vivo like microenvironment to cells in culture. The tremendous surface-area-to volume-ratio allow cells to be cultured at in vivo like cell densities. Cells are bound to a porous support, not a non-porous 2-D plastic surface, so cells do not require splitting or passaging. The molecular weight cut-off (MWCO) of the fibers helps control the composition of the micro-environment and concentrates secreted autocrine factors and biological products. The issue with MSC relative to 3-D hollow fiber bioreactors is that part of their very definition is that MSC bind to 2-D plastic cell culture flask surfaces and proliferate. However, the number of passages that can be performed before the cells differentiate is limited, 12-16 passages at most. There is no non-porous 2-D surface for MSC to attach to in hollow fiber bioreactors.

MSC cultured in hollow fiber bioreactors behave differently, and perhaps more like they do in vivo. Based upon data collected to date, MSC cultured in a 3-D hollow fiber bioreactor do not proliferate. It has not been possible to identify reliably MSC in their in vivo environment, but it is likely that the "normal" state of MSC is to be non-proliferative in vivo in the absence of specific stimuli. The 3-D environment created by a hollow fiber bioreactor recapitulates the in vivo microenvironment, and therefore MSC do not proliferate. The bad news is that MSC do not proliferate in 3-D culture, this means you require a lot of cells to seed the reactor. The good news is that MSC do not proliferate in 3-D culture. If MSC are not proliferating, they are not differentiating. Passage number is irrelevant, and cell splitting is not required. MSC have been cultured in the hollow fiber bioreactor for 30-100 days with continuous harvesting of extracellular vesicles, and no change in MSC phenotype or EV characteristics.

Guidelines for Using FiberCell C2025D, C2011, and C2018 Cartridges for MSC Culture and Extracellular Vesicle Production

Our 3-D hollow fiber bioreactors offer a natural, in vivo-like environment for MSCs, making them perfect for harvesting extracellular vesicles. Embrace this cutting-edge technology and unlock the full potential of MSC cultures for your research and therapeutic needs.

4.3.2 Fibronectin

Getting Started: Quick Attachment and Fibronectin Coating

Mesenchymal stem cells (MSCs) behave differently in a hollow fiber bioreactor compared to traditional 2-D flask culture. In the bioreactor, they tend to form spheroids instead of spreading out. Proliferation may be limited. To facilitate rapid attachment, the fibers are coated with fibronectin.

Protocol For Coating Fibers With Fibronectin:

Prepare the bioreactor cartridge (C2011, C2018, or C2025D) with PBS per the pre-culture set-up instructions.

Fibronectin Coating

To promote quick attachment and optimal growth of MSCs, it's essential to coat the fibers with fibronectin. Here's how to do it:

Step 1: Initial Setup

- Set up the C2011 or C2025 20 kDa MWCO PS cartridge with PBS, as instructed in the Quick Start Guide.
- Allow PBS to circulate in the incubator for at least 24 hours. This ensures proper cartridge equilibration and that all the PVP is rinsed off the fibers.

Understanding Fiber Coating

The polysulfone fibers in the cartridge are co-extruded with polyvinylpyrrolidone (PVP), an FDA-approved blood expansion product. PVP makes the fibers hydrophilic for easy dry storage and shipping. However, the removal of PVP renders the fibers slightly hydrophobic, allowing them to bind protein to their surface. Fibronectin, a cell attachment-promoting protein, will adhere to the outer surface of the fibers, facilitating cell attachment.

Step 2: Fibronectin Solution Preparation

Prepare a fibronectin solution with PBS. Recommended amounts are shown below. The same protocol can be used with other matrix proteins such as laminin or collagen.

C2025D 50-100 ug of fibronectin, 2 mls PBS
C2011 250ug to 1 mg of fibronectin, 20 mls PBS
C2018 1mg to 2 mgs of fibronectin, 60 mls PBS

Step 3: Fibronectin Coating of the Fibers

Follow the video instructions provided for cell inoculation into the cartridge. This process involves using two 20 mls syringes.

Load the prepared fibronectin solution into one syringe and an empty syringe into the other side port.

Close the end ports to prevent leakage through the fibers into the reservoir bottle.

Swish the fibronectin solution back and forth between the syringes approximately 4-5 times to ensure proper mixing.

After the final swish, leave half of the fibronectin solution in each syringe.

Open the right end port while keeping the left side port closed. Ultrafilter the fibronectin solution through the fibers and into the reservoir bottle.

Close the right side port after the filtration is complete and repeat the process with the left side port. The excess PBS will pass through the fibers and into the reservoir bottle, while the fibronectin will attach to the outer surface of the fibers.

Ensure both side port slide clamps are closed and both end port slide clamps are open.

Leave the cartridge at room temperature in the hood overnight to allow the fibronectin to adhere properly to the fibers.

Proceed with the rest of the preculture according to the manual.

Following this formal protocol will ensure successful coating of the C2025D, C2011, and C2018 20 kDa MWCO PS cartridges with fibronectin. This fibronectin-coated cartridge is now ready for the next steps in the pre-culture process and then cell seeding and subsequent MSC culture. Should you have any questions or require further assistance, feel free to reach out to our support team.

4.3.3 Seeding Numbers for MSCs

Proper cell seeding is crucial for successful culture. MSC and other similar cells bind to plastic flasks and spread out, occupying a lot of area per cell. When seeded into the hollow fiber bioreactor MSC will form spheroids, and not a monolayer culture. Because of this, relatively low metabolic activity of MSC, and the high capacity of a hollow fiber bioreactor it is possible to inoculate much more surface area than normally possible. We have seeded at least 2 Corning 10 stacks, or 1.32 m2 of area into the 4,000 cm2 area of the C2011 cartridge. Follow these guidelines:

- For C2011, seed a minimum of 1X108 MSCs, up to 2X109
- For C2025D, seed a minimum of 1X107 MSCs, up to 2X108
  C2018 can accommodate even higher numbers, up to $1X10^{10}$ cells

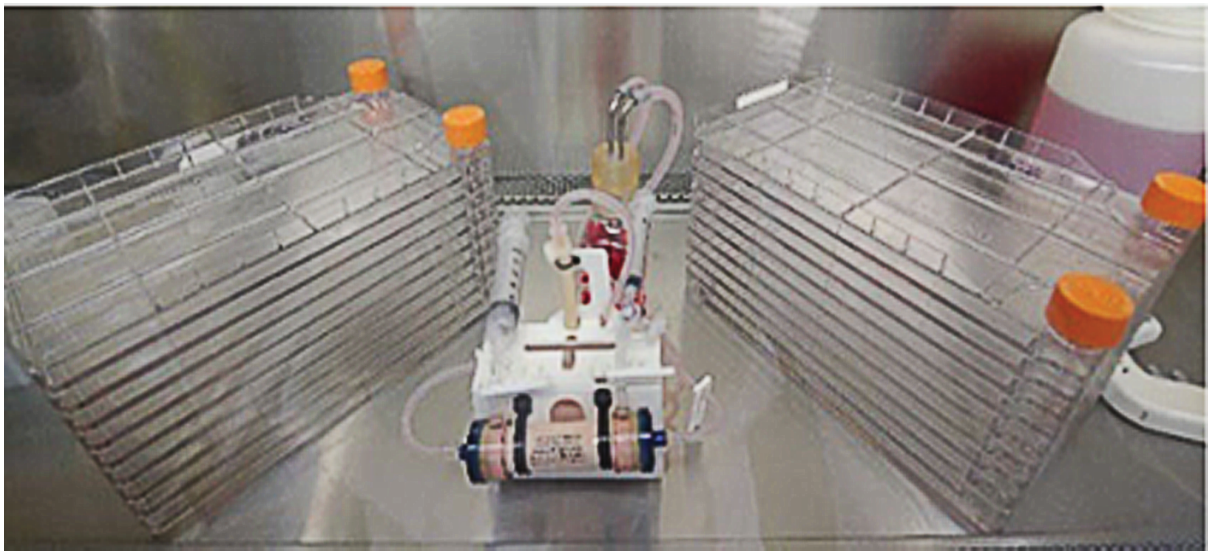

Figure 22: A large number of MSC relative to surface area can be seeded into a FiberCell hollow fiber bioreactor.

4.3.4 Choosing the Medium

Selecting the right medium is essential for MSC growth and performance. Here are some options:

Specialty xeno-free MSC mediums, which are designed to support optimal MSC growth in the bioreactor have been used with good results.

DMEM with 10% FBS or HPL (human platelet lysate) is commonly used and can provide adequate support for MSCs. This is the medium most used in our laboratories though we have used mediums from Rooster Bio and Stemcell Technology.

The most commonly used medium is DMEM with 10% FBS or HPL (human platelet lysate). Cells are seeded with medium and serum together in the ECS. After a few days of culture the FBS or HPL in the ECS is flushed out and replaced with basal DMEM only. This prevents endogenous extracellular vesicles from contaminating the ECS along with the cells, while the factors that support MSC in serum can cross the fibers. For subsequent harvests use only DMEM alone in the ECS.

Xeno-free MSC specialty mediums from various manufacturers have been used with good results. It is likely still be necessary to coat the fibers with fibronectin. Specialty attachment factor solutions, “expansion medium”, and “collection medium” of various types have not been tested in our hollow fiber bioreactors.

•

A benefit of the hollow fiber bioreactor with a 20 kDa MWCO filter fiber is that serum can be used in the circulating medium, and not with the cells in the extra-capillary space (ECS). This reduces the risk of contamination with unwanted components. It also reduces the overall protein load, simplifying subsequent filtration and purification steps.

Glucose consumption in MSCs is lower than in other cell lines grown in the bioreactor. It may be difficult to assay, but MSCs may primarily consume glutamine and produce ammonia under some circumstances. This could be related to the amount of glucose present in the medium.

4.3.5 Utilizing the Hollow Fiber Advantage For MSC Culture

The hollow fiber bioreactor’s unique 20 kDa MWCO filter fiber offers some fantastic advantages for MSC culture:

Serum can be used in the circulating medium, separate from the cells in the extra-capillary space (ECS). This minimizes the risk of unwanted contamination.

Glucose consumption by MSCs is lower compared to other cell lines. While glucose consumption may be challenging to measure glucose consumption accurately, keep in mind that MSCs may primarily consume glutamine and produce ammonia under certain conditions. This can be influenced by the glucose concentration in the medium.

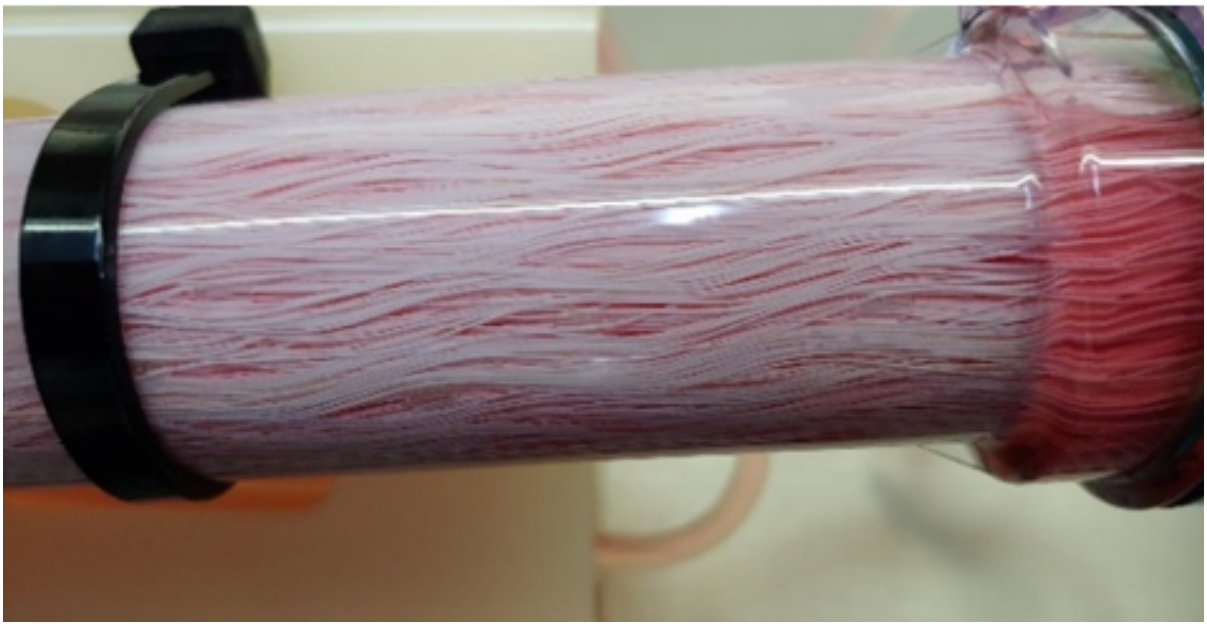

Figure 23: MSC can form spheroids when seeded into the hollow fiber bioreactor.

Harvesting EVs and MSCs from the ECS: A Few Things to Keep in Mind

Don't expect to harvest a large number of cells from the ECS of the hollow fiber bioreactor. MSCs adhere tightly to the fibers, and even spheroids bind strongly and can't be removed easily, even with trypsin.
However, you can perform a viability check:

- Harvest a small number of cells from the ECS.
- Transfer them into a T25 flask with fresh serum-containing medium. Observe for attachment and proliferation. MSCs collected from the ECS will attach and grow in a flask.

The kinetics of EV secretion by various cells is not well understood. You may see variations in EV concentrations under various conditions and harvesting intervals. We have harvested as frequently as every two hours, up to once every two days. At least once a day seems to be optimal.

Remember, these guidelines are here to help you get started with FiberCell cartridges and MSC culture in a hollow fiber bioreactor. There are many publications in the literature available. If you have any questions or need further assistance, feel free to reach out to our customer support team at **info@fibercellsystems.com and +1 301 471 1269.**

To harvest highly concentrated extracellular vesicles (EVs) from the bioreactor cartridge while safeguarding the extra-capillary space (ECS) from potential contamination, follow these steps:

(see section 3.5.3 harvesting using a 3-way stopcock)

- Attach an empty syringe to the right side-port of the cartridge.
- Switch the 3-way stop-cock so that the port leading to the sterile syringe filter is open.
- Gently tilt the cartridge upwards to drain the ECS into the opposite syringe. This process allows you to collect the valuable EVs while preventing any potential non-sterile air from entering the ECS.
- Now, it's time to replenish the ECS with fresh medium. Prepare a syringe filled with the desired medium and introduce it into the ECS in the same way as before. This ensures that the ECS is adequately filled and ready for continued EV production.
- Close the side port slide clamps securely to prevent any leaks or spills.
- Finally, turn the stop-cock so that the port leading to the .2 um sterile syringe filter is closed, and the closed end is closest to the side-port. This step provides a tight seal and ensures that the ECS remains protected from potential contamination.

By following these simple steps, you can harvest highly concentrated EVs while maintaining a sterile environment for the extra-capillary space in the bioreactor cartridge. If you have any questions or need further assistance, please don't hesitate to reach out to our support team. Happy harvesting!

4.3.6 Collecting EVs from Other Cell Types

Hollow fiber bioreactors from FiberCell Systems have been used to collect extracellular vesicles from many various cell types. Generally you can just follow the protocols for recombinant protein production. Feel free to discuss your application with FiberCell Technical Support.

4.4 Co-cultivation and 3-D Culture

Sixty years of culturing individual cell types on 2-dimensional glass or plastic surfaces using fetal bovine serum have shown it to be a powerful technique for studying cellular functions. However, cells do not grow on plastic in nature, are only exposed to serum in an activated state, and cell-matrix and cell-to-cell interactions which are not found in flasks play a key role in generating the 3-D biological organization we call multicellular "life."

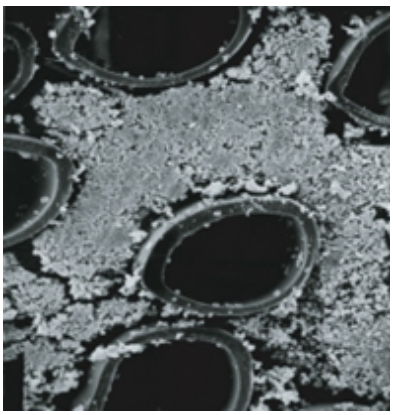

Figure 24: Cross section of hollow fibers

Current cell based assays and 2-D in vitro models are a useful, time and cost-effective tool for drug discovery and basic cell biology. Static cell-

based assays in plates, flasks or other 2-dimensional formats do not always completely mimic in vivo behavior. Hollow fiber bioreactors (HFBR) can recreate the in vivo circulatory system geometry and culture cells in a 3-D, physiologically relevant manner. They have been used to recreate many tissue/cell specific structures in a manner that allows data to be collected in meaningful ways.

Some examples of co-cultivation and 3-D models are presented below. (I find the numbers were wrong in the original file.)

1) Endothelial cell culture under shear stress
2) Endothelial cells and vascular smooth muscle
3) Cancer bio-markers
4) Cancer metastasis
5) Bone marrow and stem cells
6) Cryptosporidium
7) Plasmodium sporozoite production
8) 3-D placenta co-culture model
9) Macrophage and Tuberculosis co-culture

Hollow fiber cell culture uniquely provides a three-dimensional biomimetic cell culture environment that can recreate tissue-like structures and support co-cultivation of two or more cell types. The resulting structures permit the culture of cells and organisms not supported in 2-D flask culture. The cell numbers are high enough to enable elucidation of receptor pathways and cell signaling. Long term cultures allow for cell-to-cell interactions to fully develop, and to expose the cells and other organisms to physiological time course dependent drug dosage profiles. 3-D cultures in hollow fiber bioreactors represent a next step in in vitro tissue modelling and permits complex, in vivo models otherwise not possible.

Contact FiberCell technical support to discuss potential applications you may have in mind.

5.0 Tips and Troubleshooting for All Cell Culture Applications.

5.1 Contamination

Contamination of the cartridge with yeast, fungus or bacteria is a terminal event and the cartridge must be disposed of. There is no reliable way to clean and sterilize a hollow fiber bioreactor cartridge.The following guidelines are recommended to help prevent contamination.

A) Correct sterile technique will ensure a long a productive life for you FiberCell hollow fiber cell culture cartridge. Shortcuts, suspect medium, and careless cell culture techniques will result in a contaminated cartridge that cannot be salvaged.

B) Maintain a clean incubator. Always clean up spilled medium. Disinfect the interior of your incubator on a routine basis with a Rocall/ethanol wash or other appropriate solution.

C) Use clean distilled water with a non-volatile growth inhibitor such as Rocall (benzalkonium chloride) in the humidity pan. A humidified incubator is critical as water vapor can diffuse out of the hollow fiber cartridge oxygenation loop.

D) If supplemented growth medium is used it should be sterility tested before use.

E) The FiberCell module should be pre-cultured at maximum flow rate for 3-4 days prior to cell inoculation to check for leaks, contamination and to equilibrate the system.

F) Check to ensure that all luer fittings are securely tightened.

G) Allow bottles of cell culture medium, serum, and supplements to warm to 37°C prior to opening. The reduced pressure inside a cold medium bottle will draw in droplets of liquid and contaminants on opening.

H) Always wipe all luer fitting with an alcohol pad prior to opening. Remember, it is the evaporation of alcohol that sterilizes, not the application so allow time for the alcohol to evaporate.

I) When in doubt, use a new pipette, syringe, or bottle of medium. It is less expensive to discard or re-filter a bottle of suspect medium or pipette than to risk a contaminated culture.

J) Always use a large bore needle to draw medium or cells into a syringe. Droplets of medium from the syringe around the luer fittings invites contamination.

K) Use care and common sense.
Hollow fiber cartridges have been continuously maintained for over two years of continuous culture. Following the above guidelines will help insure a long-lived, productive culture.

5.2 Reservoir Cap

FiberCell Systems offers three different sizes of reservoir caps. 33 mm to fit onto glass media bottles with the black plastic cap, 38 mm to fit onto most standard Nalgene media bottles, and 45 mm to fit onto the wide mouth Gibco media bottles. The most common is the 38 mm reservoir cap. The length of the stainless-steel tubing can be adjusted after running the cap under some water. When you first get the reservoir cap adjust the length so that the tubes reach the bottom of a Nalgene 250 mls media bottle. This will allow you to use a 250 mls bottle to initiate the culture with 125 mls of

medium and permit the next step of 250 mls It will also allow you to use 500 mls in a 1-liter bottle. The 45mm cap reaches the bottom of a 500 mls Gibco bottle. The size of the reservoir bottle is most important for hybridoma culture.

5.3 Reservoir Size

Hybridoma culture in the hollow fiber bioreactor presents a balancing act between two cytokines 1) TGF-Beta which is inhibitory and 2) IL6 which is stimulatory. Different hybridoma clones have differing sensitivities to these two cytokines. In some cases when changing the medium and increasing the volume in the reservoir bottle during culture initiation it is useful to add fresh medium on top of the conditioned medium rather than removing and replacing 100% of the conditioned medium in the reservoir bottle. For example, after the first 125 mls of medium has its glucose depleted by 50%, add 125 mls of fresh medium to the existing 125 mls for a final volume of 250 mls.

5.4 Harvesting

- Do not start harvesting until you have a glucose uptake rate of 1 gram per day and the first liter of medium has been consumed.
- It can take 3-5 flushes of the ECS to remove most of the serum proteins after switching to CDM HD.
- Gauge the number of cells to remove, and the type of harvest(s) to perform (low glucose rate, high glucose rate or both) based upon the glucose uptake rate. With the C2011 do not allow the glucose uptake rate to get above 2 grams per day, with the C5011 do not allow the glucose uptake rate to get above 4 grams per day.
- Harvest every other day to allow antibody to concentrate in the ECS.
- One of the most common mistakes people make is to not harvest and remove enough cells when the glucose uptake rate is too high. It is normal to have a cell pellet harvest of 2-4 mls.

5.5 Purification

- Be sure to spin down the harvests immediately after collection to remove cells from the supernatant. This will prevent antibody degradation and contamination with host cell proteins.
- When using CDM HD you may be able to simplify purification protocols as the antibody of interest will be in the range of .5 mgs/mls to 5 mgs/mls and be the primary protein present in your harvested supernatant.
- When using CDM HD avoid phosphate buffers. CDM HD contains a significant amount of free iron (there is no transferrin in CDM HD) that can

interact with phosphate buffers. If you are planning on using Protein A or Protein G for antibody purification, please check the CDM HD usage instructions for alternate buffers. See https://www.fibercellsystems.com/wp-content/uploads/2018/12/4-5-CDM-HDUsageInstructions-1.pdf.

5.6 System Problems

Flowpath won't prime.

- Check that the slide clamps on the inlet and outlet are open.
- Check that there are no kinks in the oxygenation loop of tubing.
- Ensure the reservoir bottle inlet tube is submerged in medium.

Pump doesn't work.

- Check all connections.
- Is the transformer plugged in?
- Check the current in the wall outlet.
- Contact FiberCell Systems if need be.

Air bubbles in the flowpath tubing.

- Pump the system by hand making sure that the Reservoir Bottle outlet tube is submerged and not pulling in bubbles.
- Purge the system and cartridge by hand pumping.
- Open the Reservoir Cap in the hood to equilibrate the system.
- Always use prewarmed medium.

Air bubbles at the cartridge inlet.

- Tilt the outlet side of the cartridge up and hand pump the system.
- Always use prewarmed medium.

Air bubbles in the ECS.

- This is common early in preculture. Continue to preculture and check after a few hours.
- In the laminar flow hood flush the bubbles into a syringe with medium and remove them.

- Elevate the medium reservoir bottle so that the level of medium is above the level of the ECS in the cartridge.

5.7 Medium Color

Medium is bright orange or colorless.

- Lactate concentration is too high. Replace the medium in the Reservoir Bottle.
- Change medium when glucose is 50% depleted.

Medium is bright red.

- CO2 level is too low. Check the CO2 tanks for pressure. Check the lines from the tank to the incubator.

- Check the CO2 concentration in the incubator with a Fyrite at least once a week.

ECS is yellow but the flowpath medium is red.

- Cells, cell debris, cell derived extracellular matrix proteins may have compromised diffusion across the fibers. Flush the cartridge to remove excess cells. Reverse ultra filtration, pulling the medium from the flowpath into the ECS, may also help.

- Bacterial or yeast contamination in the ECS. Remove an aliquot and examine microscopically.

Medium in the reservoir bottle or ECS is cloudy and yellow or pink.

- The system has a bacterial (yellow) or yeast (pink and cloudy) contamination. Check microscopically. If contaminated, it must be dispose of immediately. Rinse the bottle and cap assembly in a bleach solution. Clean the laminar flow hood thoroughly afterwards. Dispose of all medium used for this bioreactor.

Inlet side of cartridge is occluded with a plug of white material.

- Fungal contamination. Mycelia in the perfusing medium have collected at the inlet of the cartridge. If contaminated dispose of immediately. Rinse bottle and cap assembly in a bleach solution. Clean the laminar flow hood thoroughly afterwards. Dispose of all medium used for this bioreactor.

5.8 Additional Questions

What medium is best? How do I adapt to a serum-free medium?

For hybridoma culture at high density the simpler the medium used, the better. Cells grown at high density, i.e., 1 x 108/mL, can create their own optimum micro-environment. One important factor to be considered in choosing a medium is glucose concentration, a starting glucose concentration of at least 3.5 grams/L (Ham's F12) or 4.5 grams/L (high glucose DMEM) should be used. Glucose supplementation of RPMI (1 gram/L) does not provide a robust medium for hollow fiber bioreactor culture. Some clones, i.e. NSO, can be cholesterol dependent. In this case artificial cholesterol in serum free mediums is bound up with cyclodextrin which will bind to the silicone tubing. With cholesterol dependent cell lines, it is recommended to culture using DMEM/10% CDM HD with .5-1% FBS in the circulating medium only. The cholesterol and other nutrients can cross the fibers, but bovine IgG and other large components cannot cross the fibers.

- The medium that the cells have been growing in works best for the initial medium, provided it is high glucose.

- CDM HD is the ideal medium for most hollow fiber bioreactor applications and requires no adaptation, after the cells have reached high density in the bioreactor.

- If the cells are already adapted to a specific serum free medium you can continue to use that medium in the hollow bioreactor with the understanding that there may be some high molecular weight components that may not cross the fiber.

- Adaptation to a different medium or other cell culture conditions are best made after the cells have reached high density in the hollow fiber bioreactor module. This is defined by a glucose uptake rate of 1 gram per day or higher. At that time one can change the medium in the reservoir bottle in the following manner:

- Monitor the glucose rate to ensure that the cells are not adversely affected.

- Continue this transition until the cells have adapted to the new medium.

What is the optimum serum concentration?

- Most cells should be started with 10% serum in the medium.

- After the cells have reached ~150 – 250 mgs/L glucose/day the serum can be reduced to 7.5%. Monitor the glucose rate.
- At the next change of medium, reduce the serum to 5%. Monitor the glucose rate.

- Most cell lines will be able to adapt to 2 – 3% serum. Serum levels below this amount may support cell growth but protein secretion may be inhibited. It is not recommended to use serum levels below 2%.

- Provided the cells can be cultured at high density CDM HD is a protein-free replacement for serum in most cultures.

If the culture dies, can I re-use or re-inoculate a cartridge?

- If the culture dies a second inoculation can be performed provided the cartridge is not contaminated.

- Increase the inoculum cell number by 2 – 3-fold to provide a sufficient cell mass to adapt to the bioreactor. Reduced medium volume in the reservoir may also be helpful.

- Always flush the ECS of dead cells and cell debris prior to re-inoculation.

How long will my bioreactor cartridge last?

The all-time record is 560 days for a glioma-based culture. A hybridoma culture has been maintained for almost a year with no change in antibody affinity or specificity.

Tips for a long cartridge life

- Always wipe the Reservoir bottle cap and luer fittings with an ethanol swab during feeding and harvesting.

- Perform a reverse ultra-filtration harvest (high glucose rate harvest described in the video manual) at least once a week. This helps to keep the pores of the fibers open. Use new luer caps each time you access the ECS via the side ports.

- Use one medium bottle per culture.

- Never open a cold medium bottle – the negative pressure inside the bottle may draw in water, condensation, and microorganisms.
  Keep the hood clean and uncluttered.

- Don’t rush your bioreactor work!

Remember, as long as you don’t contaminate the cartridge, we can fix anything!

Feel free to contact FiberCell Systems with any technical questions at **info@fibercellsystems.com** and **+1 304 471 1269.**

Tips or good sterile technique in cell culture:
https://www.bionique.com/mycoplasma-resources/technical-articles/better-aseptic- technique.html

6.0 CDM HD Usage Instructions

Please refer to the video “CDM HD, Chemically Defined Medium for High Density Cell Culture: https://www.youtube.com/watch?v=ZY_P5St9VuM

Preparation Of CDM HD For Use

- Fill a mixing vessel with distilled or WFI quality water, to 90% of the desired total volume of media. For example, for one liter of CDM HD, fill the vessel to 900 mls.
- Add CDM HD to the water while gently stirring. Rinse the inside of the package with some of the remaining 10% water to remove any remaining powder.
- Finish adding remaining water to bring to a volume of 1 liter and mix until completely dissolved. This should take 15-30 minutes at room temperature.
- CDM HD should be pH adjusted to 6.8 if necessary, using 1NaOH or 1N HCL.
- Filter into a sterile container by membrane filtration, using a 0.2 micron filter.
- Store at 2-8 Ĉ for both dry and rehydrated media.
- CDM HD is provided at 33.36 grams to make one liter.

Figure 25: Materials required for rehydration of CDM HD.

Use of CDM HD to Enhance EV Collection from Flask Cultures

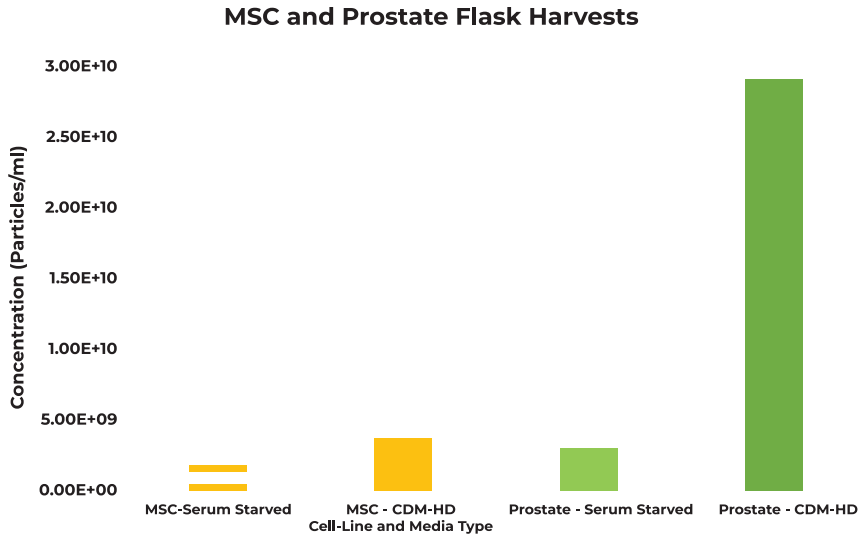

Figure 26: Harvests were taken from confluent T175 flasks with roughly 2.5×106 total cells in each flask. Serum starved flasks were grown up in 10% FBS+DMEM and serum starved for 48 hours prior to collection. CDM HD media flasks were grown up in 10% FBS+DMEM and switched to 10% CDM HD+DMEM for 48 hours prior to collection. Each flask contained 35 mls of media. Particle concentrations are as follows (particles/mls): MSC-SerumStarved (4.6×108), MSC-CDM HD (3.7×109), Prostate-Serum Starved (3.0×109, Prostate-CDM HD (2.9×1010).

Protein Purification Using CDM HD

Since CDM HD is protein free you may be able to simplify your purification protocols. This should be based upon your own direct experience along with an analysis of the harvests from your particular cell line. When purifying monoclonal antibodies from cell culture supernatants using Protein A, you should be aware that there is a significant amount of free iron present to compensate for the lack of transferrin (a protein) in the medium. This amount of iron can sometimes cause precipitation if the harvested supernatant is placed directly into a phosphate buffer system such as might be used for protein affinity purification. There are some alternative binding buffers that do not contain phosphate that can be used with Protein A. FiberCell Systems has been provided some specific information by Bio-Rad Laboratories on an appropriate binding buffer and Protein A kit that is compatible with CDM HD. Products from other companies may also be used.

One option would be to add Bio-Rad's MAPS binding buffer to enhance binding of the antibody to the Protein A gel. This buffer is optimized for mouse iG2A, but works well with most other antibodies (except rat).

Buffer formulations:

- Binding Buffer: 3.2 M NaCl in 1.6M glycine, pH9
- Elution Buffers: 100 mM Na citrate, pH3
- Regeneration Buffer: 50% methanol/water

The MAPS binding buffer is used by either adding the dry salts to a dilute sample, or it can be dissolved in water and then add two parts buffer to one part sample.

Catalog numbers for the binding buffer:

- 153-6161, buffer salts to make 5 liters
- 153-6159 Affi-Gel Protein A MAPS II kit, which includes 5 mls Protein A, an empty column, and buffers to purify 500 mgs of antibody

Bio-Rad also offers two other Protein A supports, which are pressure stable and can be run on a chromatography system.

Feel free to contact Bio-Rad technical support:
Tel: 1-800-424-6723, option 2
Email: lsg_techserv_us@bio-rad.com.

NOTES

- ***Do not heat CDM HD to speed dissolution.***
- ***H should be adjusted to no higher than 6.8 for best storage. CDM HD is stable as a liquid at 4 degrees for at least 6 weeks.***
- ***Do not freeze CDM HD once it has been reconstituted.***
- ***CDM HD powder should be stored at 4 degrees C and has a shelf life of three years from the date of manufacture.***
- ***Add CDM HD to classical media such as DMEM High glucose at a final concentration of 10%.***
- ***Store at 2-8˚C for both dry and rehydrated media.***

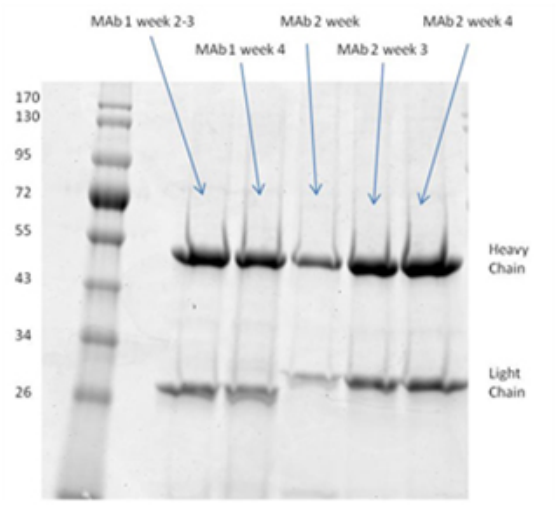

Figure 27: Unpurified Hybridoma Supernatant

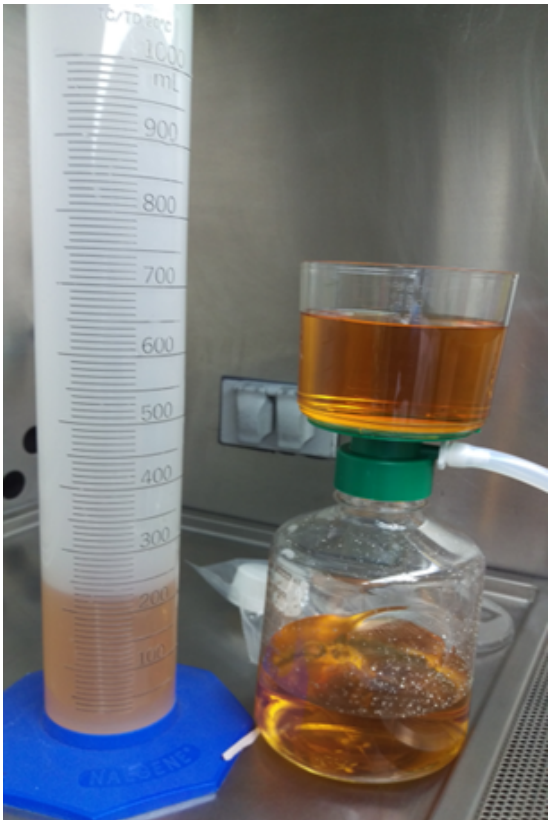

Figure 28: Sterile Filtered CDM HD

7.0 Duet Pump Operating Instructions

Please refer to the video **"Operation of a FiberCell Systems Duet Pump" https://www.youtube.com/watch?v=qA65y_3qNN4**

Thank you for purchasing a FiberCell Systems Duet pump. The Duet is designed to work with either one or two cartridges. The Duet will deliver a maximum of 220 mls/min flow rate utilizing a unique positive pressure displacement mechanism that produces no wear on the pump tubing. Low voltage power (24 volts, single phase AC) is delivered to the unit inside the incubator via a thin cord that is designed to fit through the rubber incubator door gasket. The motor is designed to emit little heat and no ozone. Operation of the duet is simple.

1. Flow rate is controlled by position of the electronic speed control knob.

    - A position of 15-20 should be used for pre-culture and the first few days of culture, 20-26 for culture and production and 26-30 for highest density culture with the C2018 and C2003 cartridges.

Figure 29: Control Box

2. Ensure that the brown pump tubing is aligned vertically in the area between the pump piston and pump platen. For maximum flow rate it is essential that this tubing be inserted properly. The flowpath should be inserted so that the pin holds it in place.

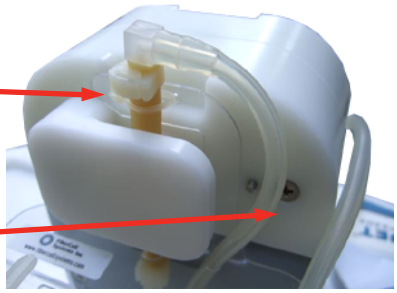

Figure 30: Pump tubing

3. The cartridge can be inserted more easily while the pump is operating.

4. Check to see that the rectangular alignment slot in the bottom of the flow path is inserted over the alignment block on the base of the Duet. It will click into place. Proper alignment is essential for optimum performance.

5. Up to a 2 liters plastic bottle can be placed into the indentation on the base of the Duet.

6. The cartridge can be inserted and removed more easily while the pump is in operation. It is also easier to leave the Duet pump in the incubator and simply remove the cartridges rather than take the Duet pump into the incubator.

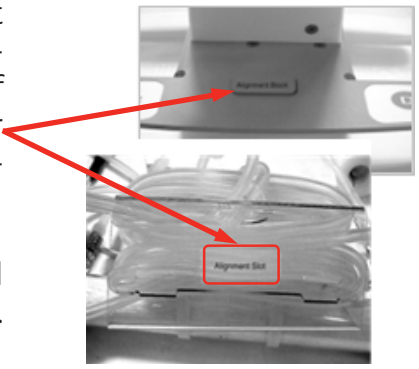

Figure 31&32:
Rectangular alignment slot.

Precautions

- Do not insert fingers or delicate objects between the pump pistons and pump platens.
- Do not leave the pump inside an incubator unless the motor is running or rust may occur.
- Do not wipe the unit with anything stronger than 70% ethanol. Do not use bleach or other strong solvents. Do not autoclave the unit.
- Keep the unit plugged in to a surge protector or battery back-up power source.
- Do not put the flow rate controller into the in cubator. It will attach to the side of most incubators using the magnets on the back of the control box.

Figure 33:
Thin cord for power fits
in between incubator door.

Flow Rate and Hollow Fiber Cell Culture

The rate limiting factor for most cells cultured in a hollow fiber bioreactor is the oxygenation level. Oxygenation is provided by recirculation of medium through the loop of silicone tubing on the back of the flow path, silicone tubing is quite gas permeable and FiberCell Systems uses a thin wall silicone tubing for optimum gas exchange. Nutrition delivery is not flow rate dependent to the degree that gas exchange is. It really is not possible to have too high a flow rate though there can be too low of a flow rate. It is best to start the cultures at a flow rate of 15, this will provide plenty of flow rate and gas support without generating such a high pressure wave that cells will not attach to the fiber. After three days of culture increase the flow rate to 25. Flow rates higher than that should only be used at glucose uptake rates of 2 grams per day or higher. Do not use a flow rate setting of greater than 10 with the C2025D cartridge.

Figure 34: Duet Pump fits in the standard incubator.

Your Duet comes with a two-year full warranty when registered using the FiberCell Systems web site. For technical support, contact FiberCell Systems at **(301) 471-1269** or e-mail at **info@fibercellsystems.com.**

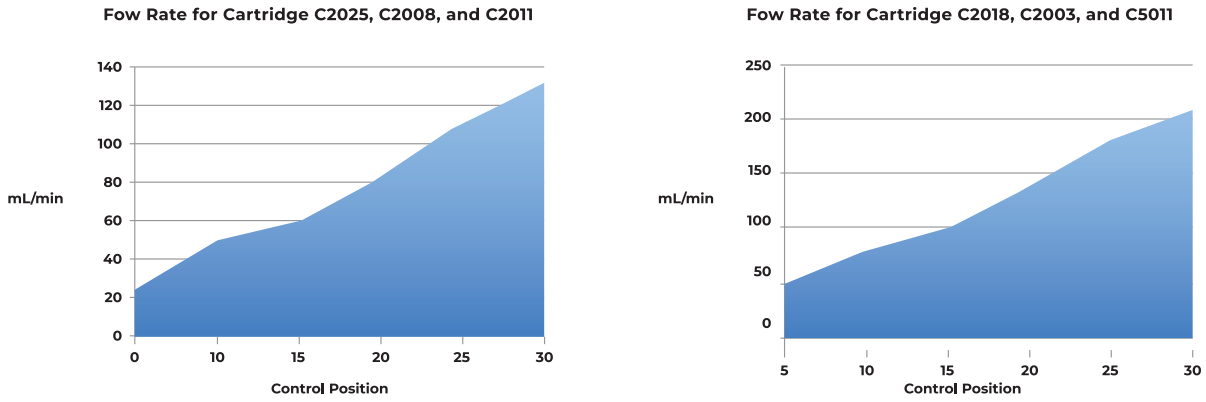

Figure 35: Duet flow rates vs. control knob position.

Specifications

- Operating temperature: 5-40 C
- 10-100% humidity
- Altitude up to 3,000 meters

- Transportation and storage temperatures -10C to +55C
- Current draw 500 mA
- Do not autoclave or immerse in liquid. Clean by wiping with a solution- of 70% ethanol.
- Do not open, no user serviceable parts. Disassembly of any kind will void the warranty.
- Warranty: Full two years parts and labor.
- Dimensions 23cmX43cmX18cm
- Weight: 4.2 kg
- Airborne noise emissions:
  1) A-weighted emission sound level does not exceed 70db (A)
  2) Peak C-weighted instantaneous sound pressure does not exceed 63 Pa
  3) A-weighted sound power level does not exceed 80db(A)
     No ionizing radiation emitted.

8.0 Appendix – Additional Lab Supplies and Vendors

Sterile Male/Female Luer Plug	Medex (800) 848-1757 (614) 876-0375 FAX catalog number #MX-491
Luer Lock Syringes	All major medical distributors 10cc, sterile, individually wrapped Specify syringes with Luer-Lok tips E.g. B-D #309604
Glucose Measurement	Cesco Engineering Glucell meter
2 and 4 liter medium bottles	United States Plastics catalog number 72004, 72005 1390 Neubrecht Rd. Lima, Ohio 45801-3196 Phone: 1-800-809-4217 Fax: 1-800-854-5498 www.usplastic.com

Clave fittings	ICU Medical **https://www.icumed.com/products/infusion-therapy/iv-consumables/clave-iv-connector-technology**
Autoclavable Plastic medium bottles	Tri-Forest Labware **https://www.triforestlabware.com**

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User Manual & Quick Start Guide