Endothelial Cartridge Instructions

FiberCell Systems® Inc., A Better Way To Grow Cells
168 West Main Street, #922, New Market, Md. 21774
www.FiberCellSystems.com, To Order Call 301-471-1269 or 240-440-2662
Revision 6.2 2/11/2022

Table of Contents:

1) Introduction……………………………………………………3
2) Culture Guidelines…………………………………………..… 4
3) Overview……………………………………………………..… 4
4) Activating the Endothelial Cartridge……………………..… 5
5) Loading the Endothelial Cartridge ...……………………...... 7
6) Daily Maintenance Schedule ………………………………….. 8
7) RNA Isolation from Endothelial Cells……………………….. 9

Introduction

Thank you for your purchase of a hollow fiber bioreactor system from FiberCell Systems. A hollow fiber bioreactor cartridge will allow you to culture more cells, produce more protein and antibody at a higher concentration and in a smaller space than is possible with any other culture method. Because the cells are growing at 100X density than other techniques there will be some methods that are counter-intuitive to the ways that you may currently be growing cells.

These products are for laboratory use only. Not for diagnostic or therapeutic use in humans or animals.

Read the entire FiberCell Systems User's Manual prior to use. This provides important information on system set-up, maintenance, and daily monitoring of hollow fiber cultures. This manual is available from our web site at www.fibercellsystems.com.

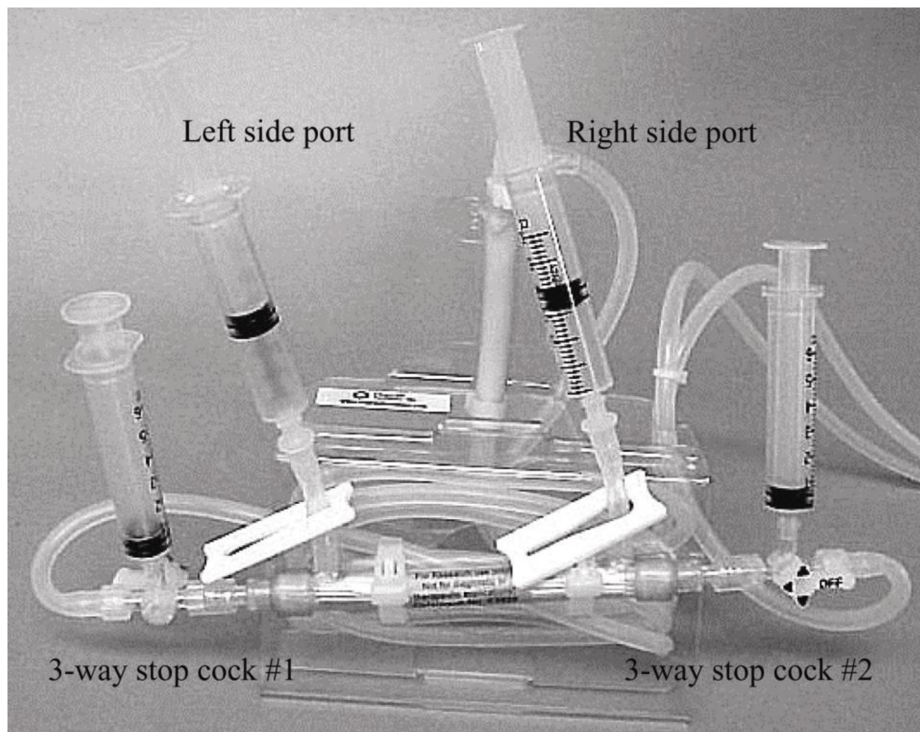


Photo: FiberCell Systems cartridge with ports identified.

Culture Guidelines

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 invites contamination.
- Perform all operations in the laminar flow hood. Keep the hood clean. Avoid rapid movements and working directly over the samples. If it necessary to open the hood front be sure to allow time for the air inside the hood to completely exchange.

Overview

The FiberCell Systems Polysulfone Plus cartridge (cat# C2025) contains a unique hollow fiber manufactured from a material that allows the hydrophobic binding of proteins to the fiber matrix. It is only necessary to wet out the fiber using 70% ethanol/water to activate the fiber and allow proteins to attach. This protein attachment is of the order of 10µg to 100µg per cm^2 of fiber area (70cm^2 for C2025). The fiber pore size of this material is .1µm so the protein coating will be uniformly applied to both the inner and outer surfaces of the fiber. Applications for this fiber include the study of the effects of extra-cellular matrix (ECM) composition on the growth of cells such as hepatocytes, pancreatic islets and other cell types where ECM composition may affect cell growth and differentiation. The FiberCell Systems Polysulfone Plus cartridge allows for the long term study (weeks to months) of ECM matrix on cell growth and development.

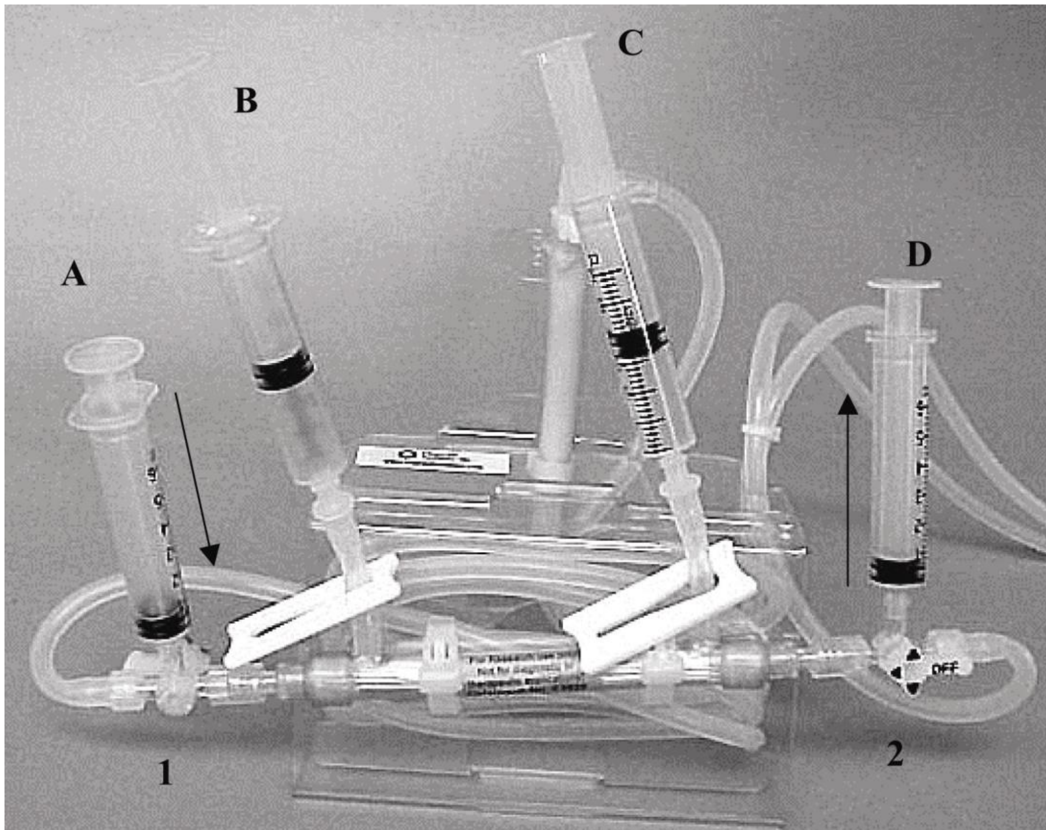
Another application for the Polysulfone Plus fiber is for the culture of endothelial cells under conditions of chronic shear stress. They are designed to be coated with appropriate matrix proteins such as fibronectin, collagen, gelatin or other proteins that facilitate the attachment of endothelial cells when inoculated onto the inside wall (lumenal wall) of the fiber. A solution of 10% FBS in standard cell culture medium can also be used. Endothelial cells attached in this manner can be subjected to various levels of reproducible shear stress for long term culture, up to 28 days or more. When grown under these conditions of chronic shear stress endothelial cells behave very differently than when grown in static culture. Endothelial cells will lay flat, form a monolayer and orient to the direction of medium flow forming tight junctions. Physiologic expression of Palade bodies can be observed and some genes can be expressed that are not expressed in static culture. Culture of endothelial cells under chronic shear stress is considered to be a more physiologic environment and closer to

the *in vivo* ideal. It is also possible to set up the FiberCell Systems Polysulfone Plus cartridge as a co-cultivation system in which endothelial cells are loaded into the lumen of the fiber and other cells types such as vascular smooth muscle or astro-glial cells are placed outside of the fiber. Examples of this can be found in the references included in the FiberCell Systems User's Manual.

Proper sterile technique is essential to maintain the long term health of this culture system. The FiberCell Systems materials used in the construction of this hollow fiber permits the easy application of various protein matrices such as collagen, fibronectin or gelatin. Also, it is possible to bind cytokines in conjunction with the proteins such as VEGF (vascular endothelial growth factor). Recovery of intact endothelial cells can be variable depending upon cell line and culture conditions. However, recovery of RNA and proteins can be achieved easily. Various microscopic techniques such as SEM, TEM, and immunohistochemical staining can be applied. Growth of endothelial cells under chronic shear stress using the FiberCell Systems' Polysulfone Plus cartridge is the most physiologic way to culture and study endothelial cell growth and function.

Set up of this system requires three steps. The first is to activate and coat the cartridge with the proteins of choice. The second is to load to endothelial cells into the cartridge. The third is to adapt the endothelial cells to the desired shear stress levels.

Activating the Endothelial Cartridge



1. Prepare the following items in the laminar flow hood
   - 6 each 5 or 10ml syringes

   - 10-20 mls 70% ethanol/water sterile filtered
   - 10-20 mls sterile PBS

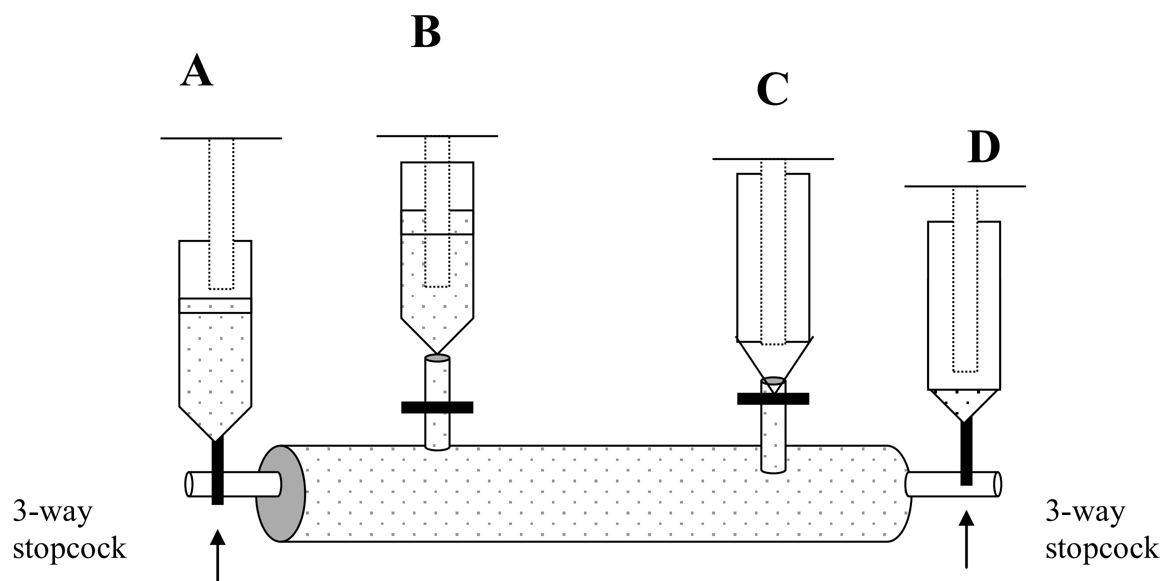
   - 10 mls sterile water

   - 5-10 mls of coating solution, 1mg/ml in sterile PBS. This solution can be fibronectin, collagen, gelatin, serum or other appropriate matrix proteins.

2. Fill a syringe with alcohol solution and attach it to 3-way stopcock #1. Position stopcock so that the "off" is facing away from the cartridge. Flow should go from the syringe into the cartridge.
3. Attach an empty syringe to 3-way stopcock #2. Position the "off" so that it is facing away from the cartridge. Flow should travel from the cartridge to the syringe.
4. Side port slide clamps associated with syringes B and C should be closed.

5. Flush alcohol from syringe A to syringe D. The ethanol should be in contact with the fiber for at least one minute. You will be able to see the fiber "wet out".
6. Drain any excess ethanol from the cartridge using syringe D.
7. Fill a fresh 10ml syringe with sterile distilled water. Attach to stopcock #1
8. Empty syringe D of ethanol and re-attach.
9. Rinse cartridge with sterile water. The water should be in contact with the fiber for at least 60 seconds. Flush back and forth between syringes A and D to remove alcohol. Remove excess sterile water using syringe D.
10. Fill a new syringe with protein matrix solution and attach to 3-way stopcock #1.
11. Attach a new, empty syringe to 3-way stopcock #2.
12. Attach new empty syringes to side ports B and C. Open side port slide clamp C
13. Position 3-way stopcock #2 so that the "off" is facing the cartridge.
14. Flush protein solution from syringe A to syringe C through the fiber. Flush solution back into syringe A.
15. Close side port slide clamp C. Position 3-way stopcock #2 so that the "off" is facing away from the cartridge. Flush protein solution from syringe A to syringe D.
16. Open side port slide clamp B. Position 3-way stopcock #1 so that the "off is facing away from the cartridge.
17. Flush protein solution from syringe D to syringe B and back to syringe D.
18. Ensure that the cartridge is filled with protein solution. Close both side port slide clamps and position the two 3-way stopcocks so that the "off" is facing towards the cartridge.
19. Let protein solution coat the cartridge for a minimum of one hour.
20. Position 3-way stopcocks so that the "off" is facing the syringes. Remove the syringes and replace with sterile luer caps. Initiate pre-culture for 24 hours.

Loading Endothelial Cells



21. Prepare the following items in the laminar flow hood
    - 4 each 5 or10ml syringes
    - Freshly harvested endothelial cells from 2 T75 flasks in a volume of 10mls complete medium.
    - 18 gauge or larger needle
    - 4 sterile male luer caps
22. Attach a syringe to 3-way stopcock A and side ports B and C.
23. Using the large gauge needle draw 10 mls of the endothelial cell suspension into the 4th syringe. Remove the needle and attach the syringe to 3-way stopcock D
24. Side port slide clamps B and C should be closed. 3-way stopcocks A and D should have the "off" position facing away from the cartridge
25. Gently flush the cell suspension between syringe A and D through the inside of the cartridge 3 to 4 times. Leave half of the cell suspension in each of the syringes when finished
26. Open side port slide clamp C. Turn 3-way stopcock D so that the "off" position is turned towards the cartridge.
27. Slowly transfer medium and cells from syringe A to syringe C. Excess medium will flow through the walls of the fibers and the endothelial cells will be trapped in the lumen of the fibers.
28. Close side port slide clamp C and turn 3-way stopcock A so that the "off" position is turned towards the cartridge.
29. Open side port slide clamp B and turn 3-way stopcock D so the "off" position is turned away from the cartridge.
30. Slowly transfer medium and cells from syringe D to syringe B. Excess medium will flow through the walls of the fibers and the endothelial cells will be trapped in the lumen of the fibers.
31. Close side port slide clamp B and turn both 3-way stopcock A and D so that the "off" position is facing towards the cartridge.
32. Place the endothelial cell culture cartridge into the incubator for one hour WITHOUT flow to allow the endothelial cells to attach to the fiber. Rotate the cartridge 180 degrees after 30 minutes.
33. After the one-hour attachment period in the incubator return the cartridge to the hood. Remove the syringes and replace with sterile luer caps. Turn the 3-way stopcocks so that the "off" position is facing towards the position where the syringes were attached and will allow medium to flow through the cartridge.

Place the cartridge into the FiberCell Systems pump system and initiate flow at the lowest rate (30 pulses per minute) and shortest stroke length for approximately 12-24 hours. Check that the pump tubing is being compressed by the pump platen as the pre-culture period can occasionally distort the tubing slightly. Restore the original shape by GENTLY squeezing the tubing back into shape. This level of medium flow is sufficient to provide oxygen and nutrients to the endothelial cells without generating a level of shear that will remove the cells from the fiber. After 12 hours change the medium in the reservoir bottle. Retain the initial medium and count the cells that did not attach to the fiber. This will provide a direct indicator of the number of cells that attached to the fiber. Typically 20-40% of the cells loaded will not adhere. If desired the endothelial cell inoculation can be divided into two equal portions and the loading steps repeated.

Daily Maintenance Schedule

Day 0: Start the newly inoculated cartridge with 50mls of medium in the reservoir bottle. The will allow the accumulation of cytokines during the first few days of culture. The flow rate should be set to low, position 1 on the FiberCell Systems pump. The cartridge should remain at this flow rate for the first day of culture. Check to insure that the pump tubing has become distorted during the pre-culture period. If it is not being compressed restore the circular shape of the tubing by squeezing it gently.

Day 1-7 Adapt the cells to shear stress by increasing flow rate no more than one position increment at a time. Once the cells have been adapted to position 5, low rate it is possible to increase the rate to high and gradually select higher shear stress levels based upon the flow rate and shear stress levels in the chart below.

Check the reservoir bottle for endothelial cells that have washed from the cartridge. This will give an indication of the cells remaining in the cartridge.

Change the cell culture medium when the glucose has been 50% depleted.

Flow rate and Shear Stress

$$\tau = (4\eta Q/\pi R^3)$$

η = viscosity (dyne sec/cm^2)
Q = fluid flow rate (ml/sec) (per fiber)
R = internal radius

Viscosity of cell culture medium with 10% FBS is approximately 0.008 dyne sec/cm^2
Fluid flow rate must be converted from mls/min to mls/sec. Also, there are 20 fibers in the cartridge so this flow rate must be divided by 20

Internal radius of the fiber is 350µm (0.07 cm)

R^3 = 0.000043
1 ml/min flow rate per fiber = 0.0167

Shear stress at 20mls/minute = 3.95 dynes/cm^2

It is strongly recommended that you calibrate your pump system in your own laboratory for flow rate.

Flow	Shear
5 mls/min	1.2 dynes/cm2
12 mls/min	3.0 dynes/cm2
15 mls/min	3.75 dynes/cm2
24 mls/min	6.0 dynes/cm2
30mls/min	7.5 dynes/cm2
40 mlsmin	9.8 dynes/cm2
55 mls/min	14 dynes/cm2
70 mls/min	17.5 dynes/cm2
85 mls/min	21 dynes/cm2

RNA Isolation from Endothelial Cells

Overview: Endothelial cells are cultured on the inside wall of the hollow fiber module under conditions of defined shear stress and time. The cells are then lysed in situ using a guanidinium isothiocyanate solution. RNA is purified and quantitated using conventional molecular biology techniques.

Utilize all precautions to prevent contamination of the samples with RNase from the researcher and laboratory. All reagents should be RNase free. Volumes indicated are for one FiberCell Systems catalog number C2025 hollow fiber module.

Materials

Reagents

- TRIZOL Reagent, Invitrogen Inc. cat # 15596-026
Similar RNA isolation reagents based upon the acid guanidinium thiocyanate/phenol method of Chomczynski and Sacchi are available from other vendors
- Chloroform, (molecular biology grade, without additives)
- 75% ethanol (v/v) (molecular biology grade) in DEPC-treated (RNase-free) water

- RNase-free water or 0.5% SDS solution in RNase-free water

Supplies
- sterile pipette tips
- sterile plastic pipettes, individually wrapped
- sterile 1.5 ml microfuge tubes
- 50 and 15 ml polypropylene centrifuge tubes (12,000 X g)
- disposable latex gloves (talc-free)
- sterile 10cc luer-lock syringes

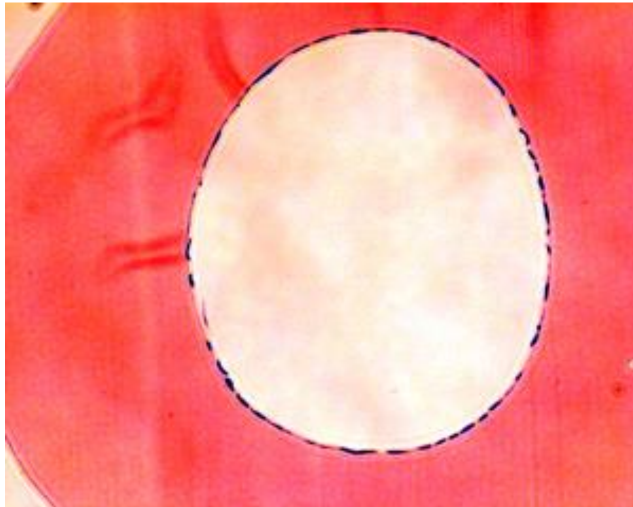
Equipment
- micro-pipettes
- refrigerated centrifuge capable of 12,000 X g
- table top microfuge (refrigeration optional)
- Speed-Vac, water bath or other method of drying RNA pellets
- -20°C freezer
- UV Spectrophotometer

Cartridge Preparation

34. Remove module from the FiberCell Systems pump and place into the laminar flow hood.
35. Attach an empty 5-10 ml syringe to one of the 3-way stop cock valves. Remove the luer cap from the other 3-way stop cock. Set both 3-way stop cocks such that the "off" position is facing away from the cartridge and so that flow will proceed from the cartridge to the syringe.
36. Drain the cell culture medium from the lumen of the fibers by gently withdrawing the medium into the syringe. Discard.
37. Using the same syringe (or a fresh one) remove the luer cap from one of the side ports and attach. Remove the luer cap from the other side port and remove any cell culture medium from the ECS. Discard.
38. Close the side port slide clamps.
39. Detach the cartridge from the 3-way stop cock valves. Attach an empty 5 or 10 ml syringe to one end of the cartridge.
40. Draw 3-4 mls of cold (4°C) TRIZOL reagent into a 5 or 10 ml syringe and immediately attach it to the other end of the cartridge.
41. Flush the lysis solution both and forth between the two syringes 7-10 times and collect into one syringe. Place lysate into a 50ml conical centrifuge tube.
42. Repeat the RNA extraction 2 more times by repeating steps 7 and 8 above twice. Collect lysate into the same 50ml conical centrifuge tube.
43. Allow the pooled samples to incubate at room temperature for 5 minutes to allow for complete dissociation of the nucleoprotein complex.
44. Follow the manufacturer's recommendations for precipitation, washing and quantitation of RNA.

C2025 Specifications

Fiber number	20
Fiber I.D.	700µm
Fiber O.D.	1,300µm
Wall Thickness	300µm
Pore Size	.1µm
Lumen Surface Area	$70cm^2$
Outer Surface Area	$85cm^2$
Inoculation cell number	$5\text{-}10 \times 10^6$
µg of Recovered RNA (est.)	50-150



Pulmonary endothelial cells grown at 5 dynes/cm^2. Photo courtesy of Dr. Guerkan Sengogle, Vienna Hospital