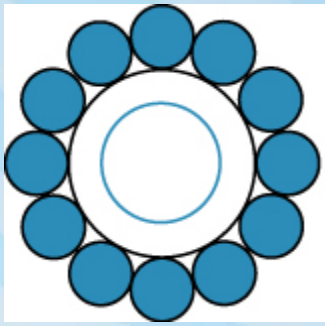

FiberCell Systems Inc.

a better way to grow cells

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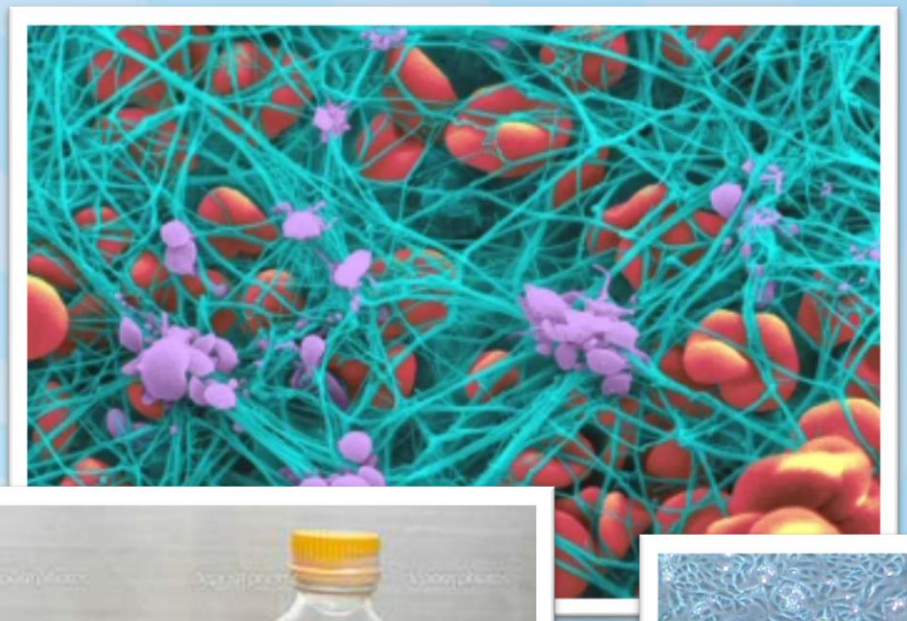
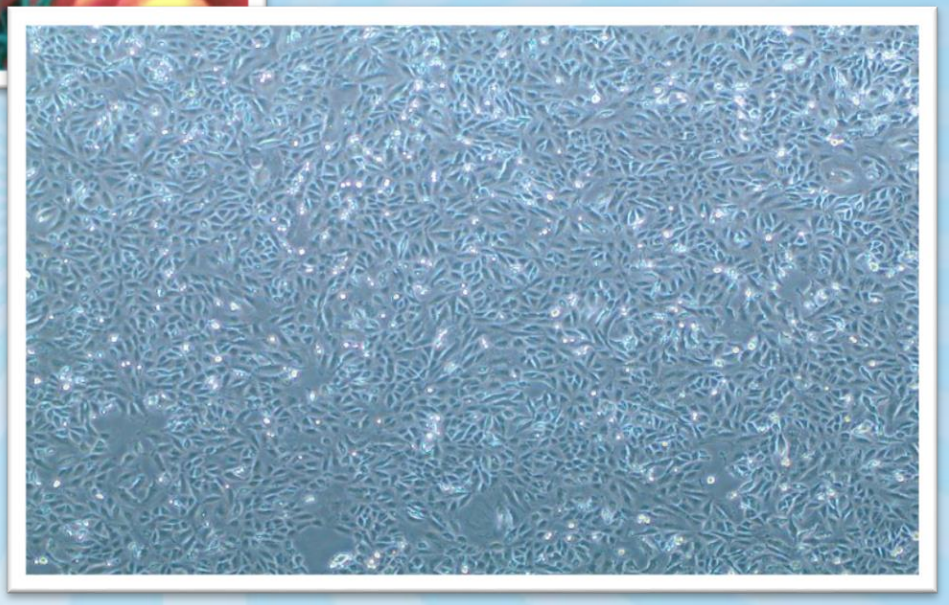
Cell Culture Through the Ages





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Cell Culture Options for Scale-Up

- Roller Bottles
- Cell Factory
- Cell Cube
- Cell Culture Bags
- Spinner Flasks
- Bioreactors

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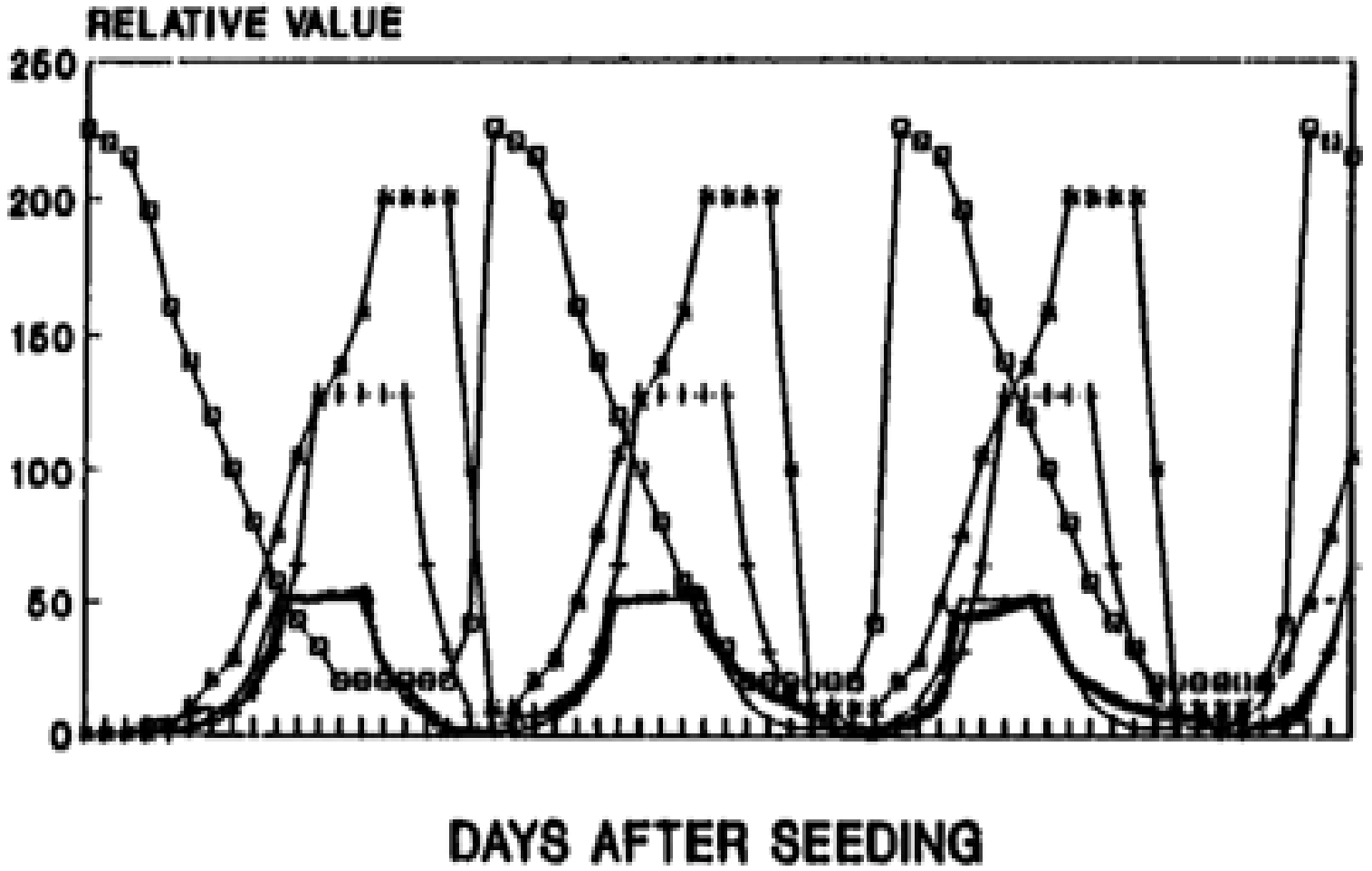
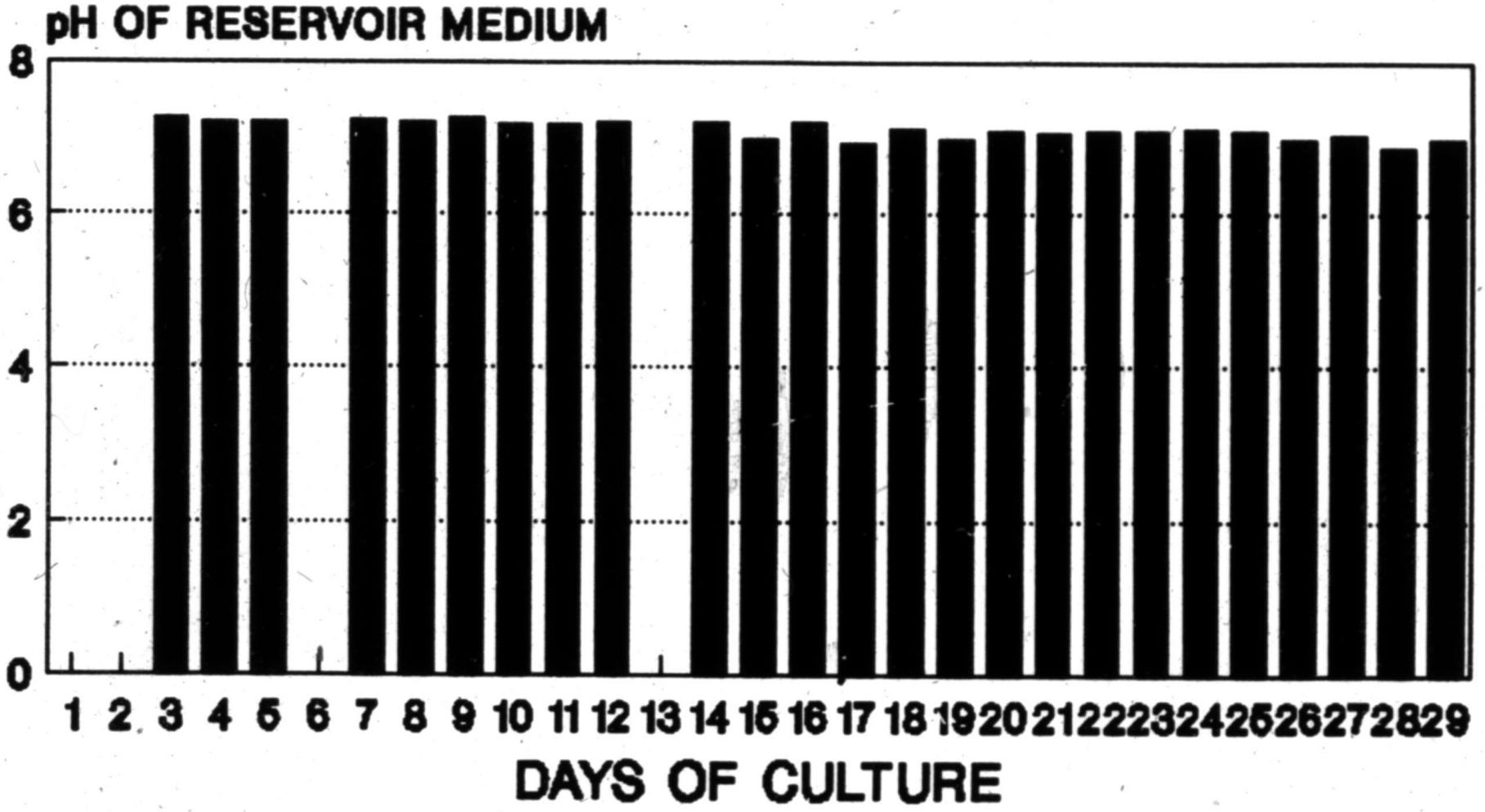
"Feast or Famine"

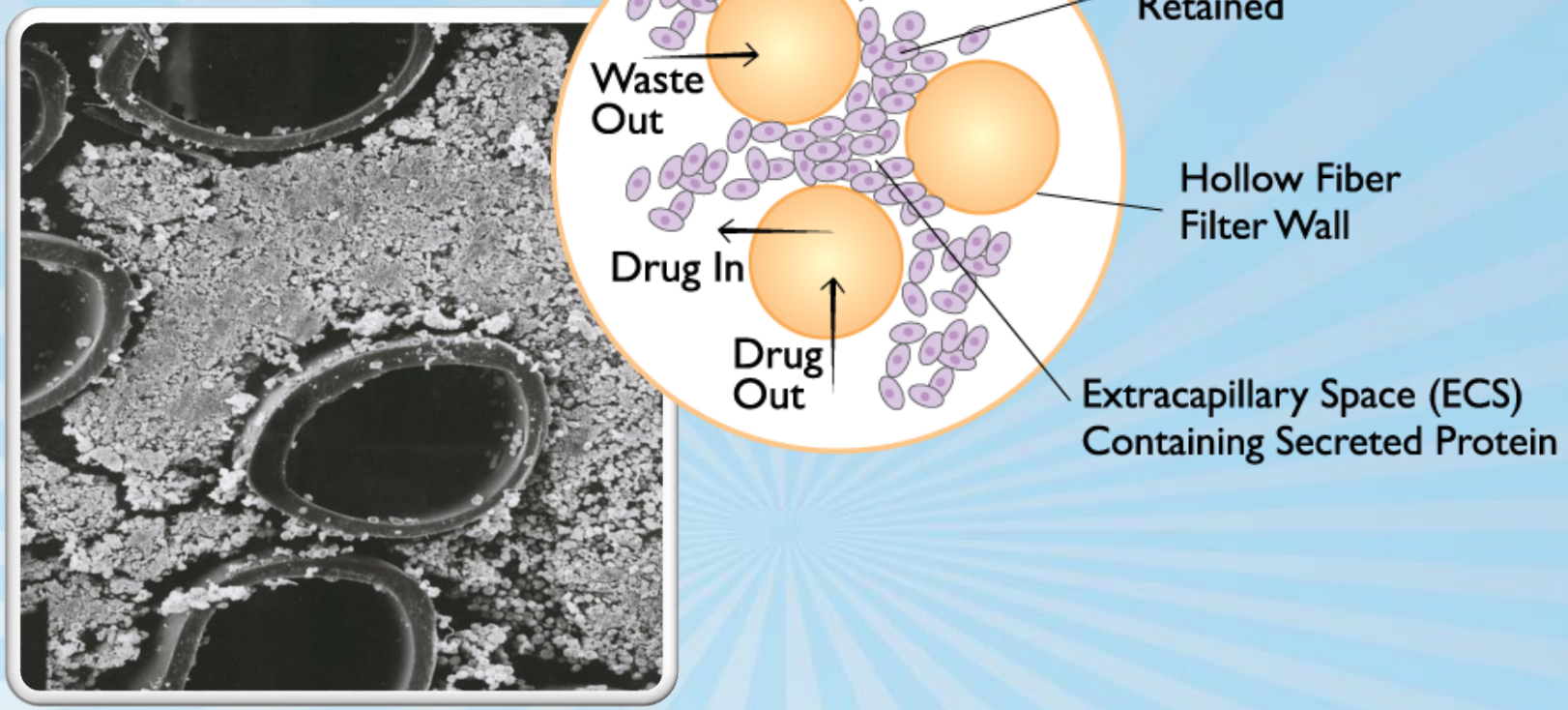


HF CULTURE OF CHO CELLS

pH CHANGES



Hollow Fiber: How it works



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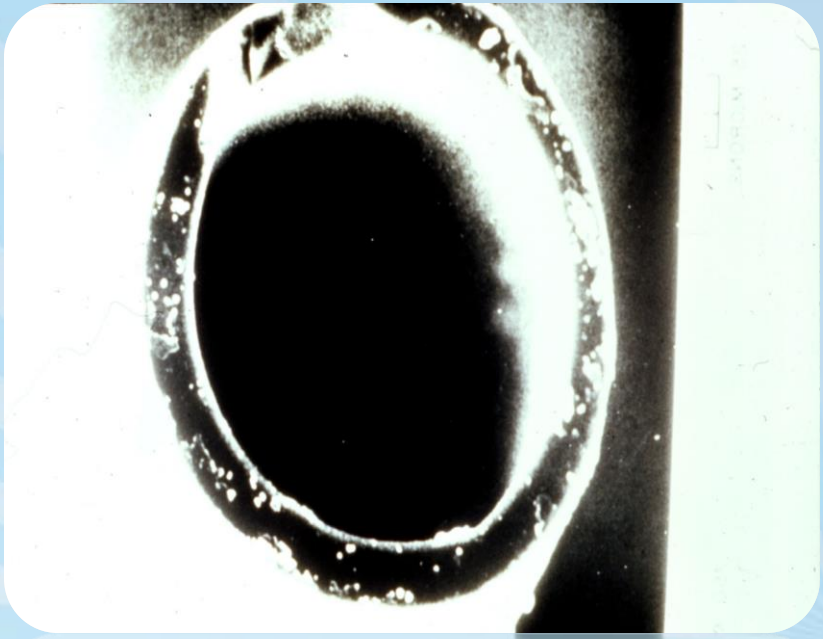
Hollow Fiber Specifications



- Hydrophilic Polysulfone or PVDF
- 210µm O.D.
- 8µm wall thickness
- GFR of 20kd fiber in excess of 140

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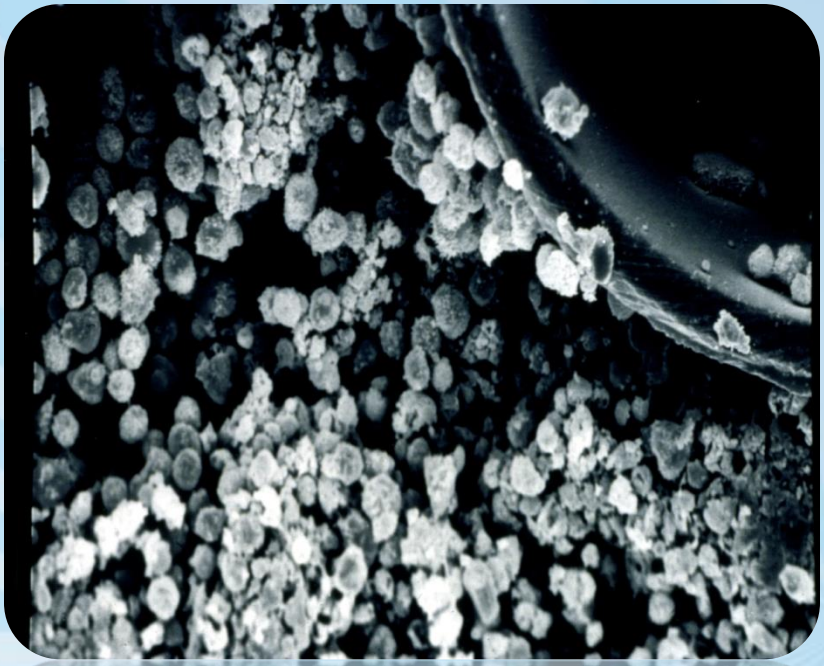
HF Culture of Lymphocytes



- 10^8 + cells/ml
- "Wavy" fibers optimized for suspension cells
- High cell density permits adaptation to lower serum concentrations and protein free medium such as CDM-HD

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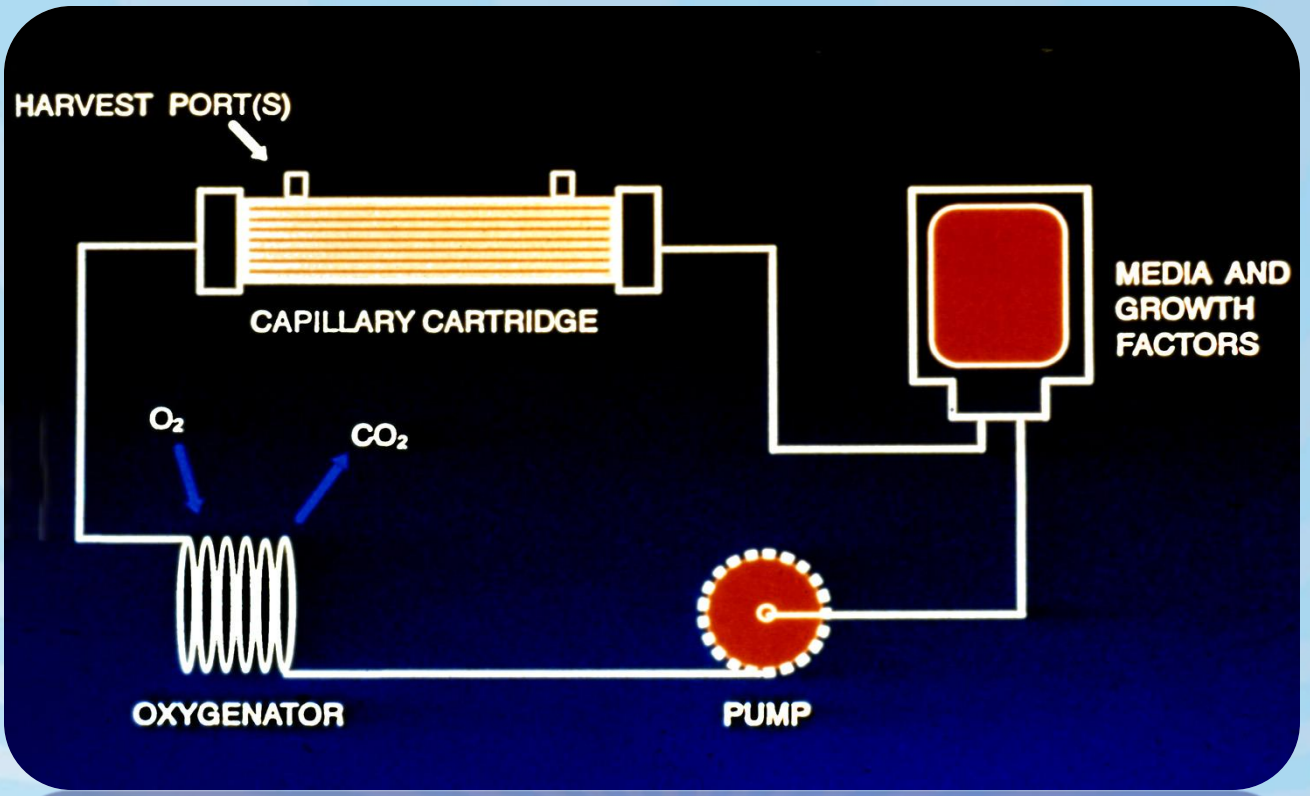

- Positive pressure displacement pump
- Silicone tubing for gas exchange
- Closed, bio-safe system

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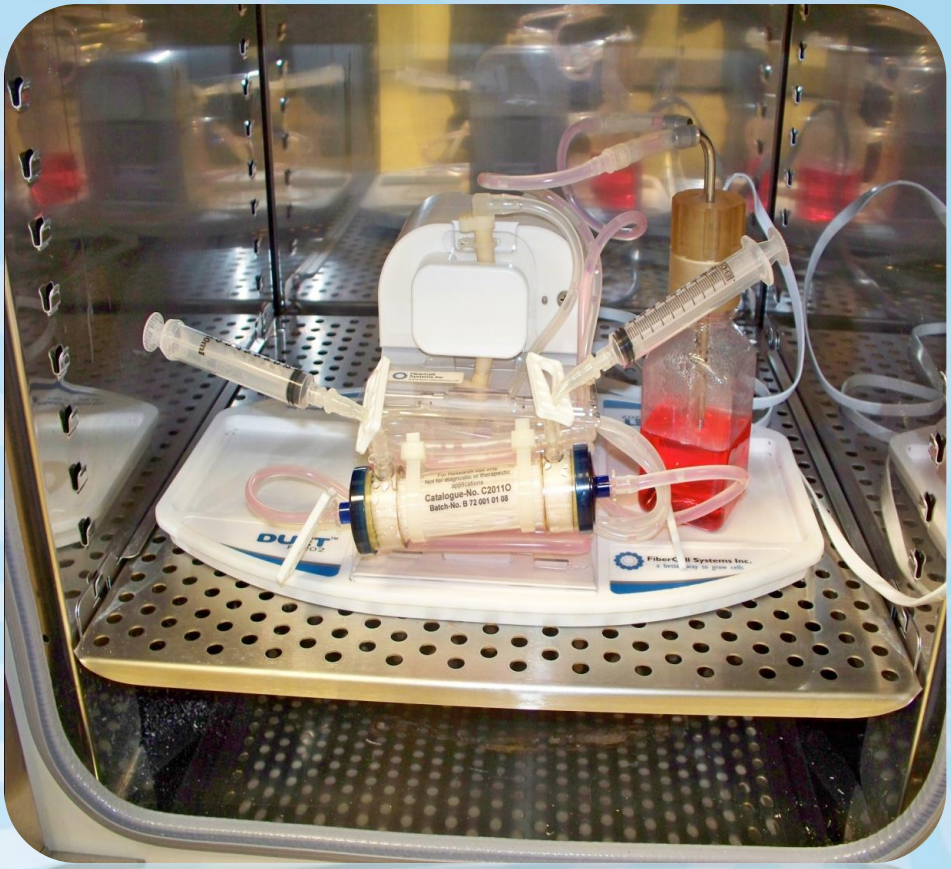
In the Laboratory



- Fits in any standard sized incubator
- Gas controlled by incubator
- Temperature controlled by incubator
- Thin cord for power

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Working with the cartridge

- Moves easily into hood
- Good sterile technique always a plus
- Maintenance only 15 minutes per day
- Harvest product and measure glucose consumption

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HF Applications

- Monoclonal antibody production
- Recombinant protein production
- Conditioned medium
- Exosome production
- Endothelial cell culture under shear stress
- Cell co-cultivation
- Virus production
- *In Vitro* toxicology

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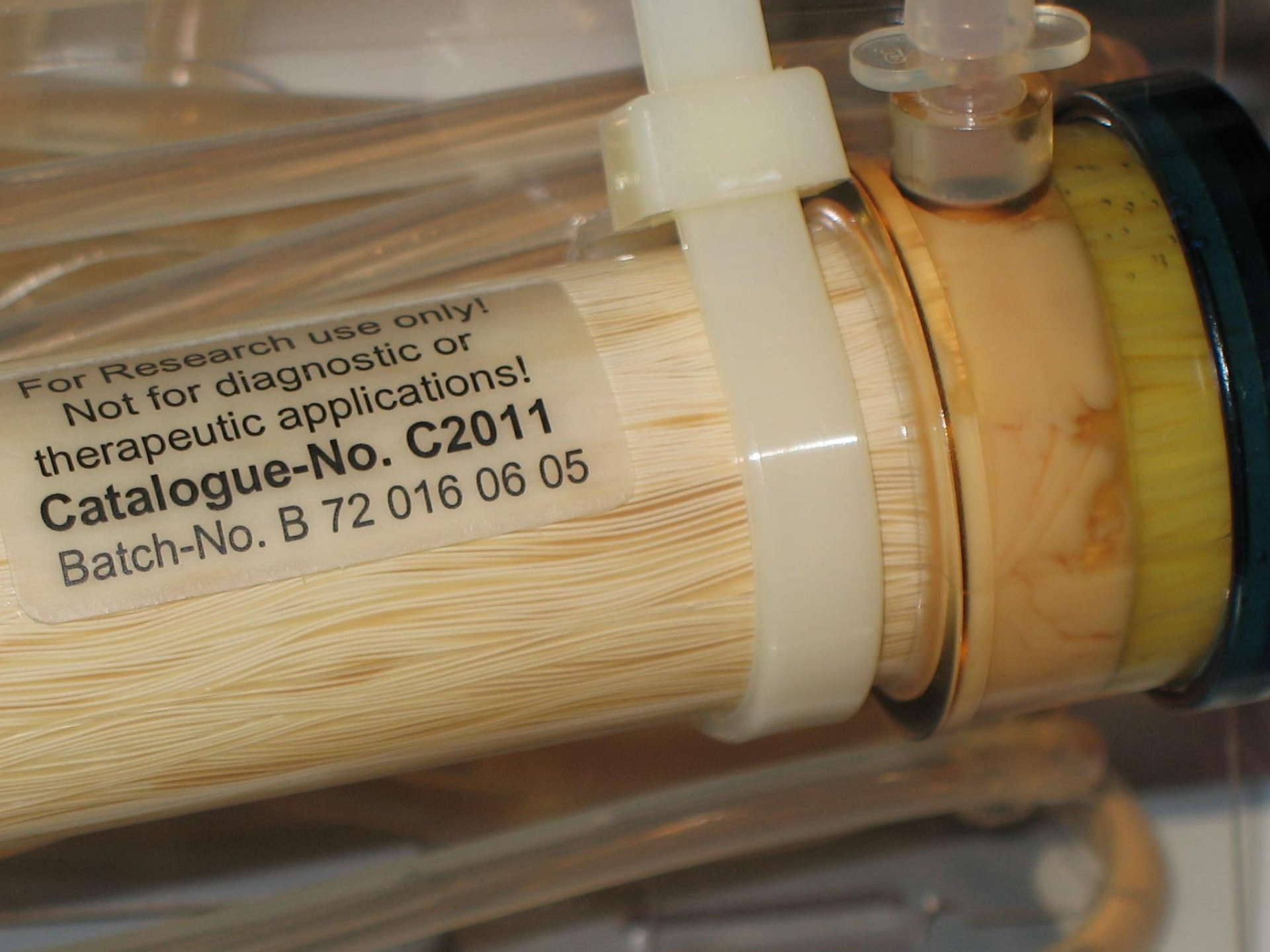
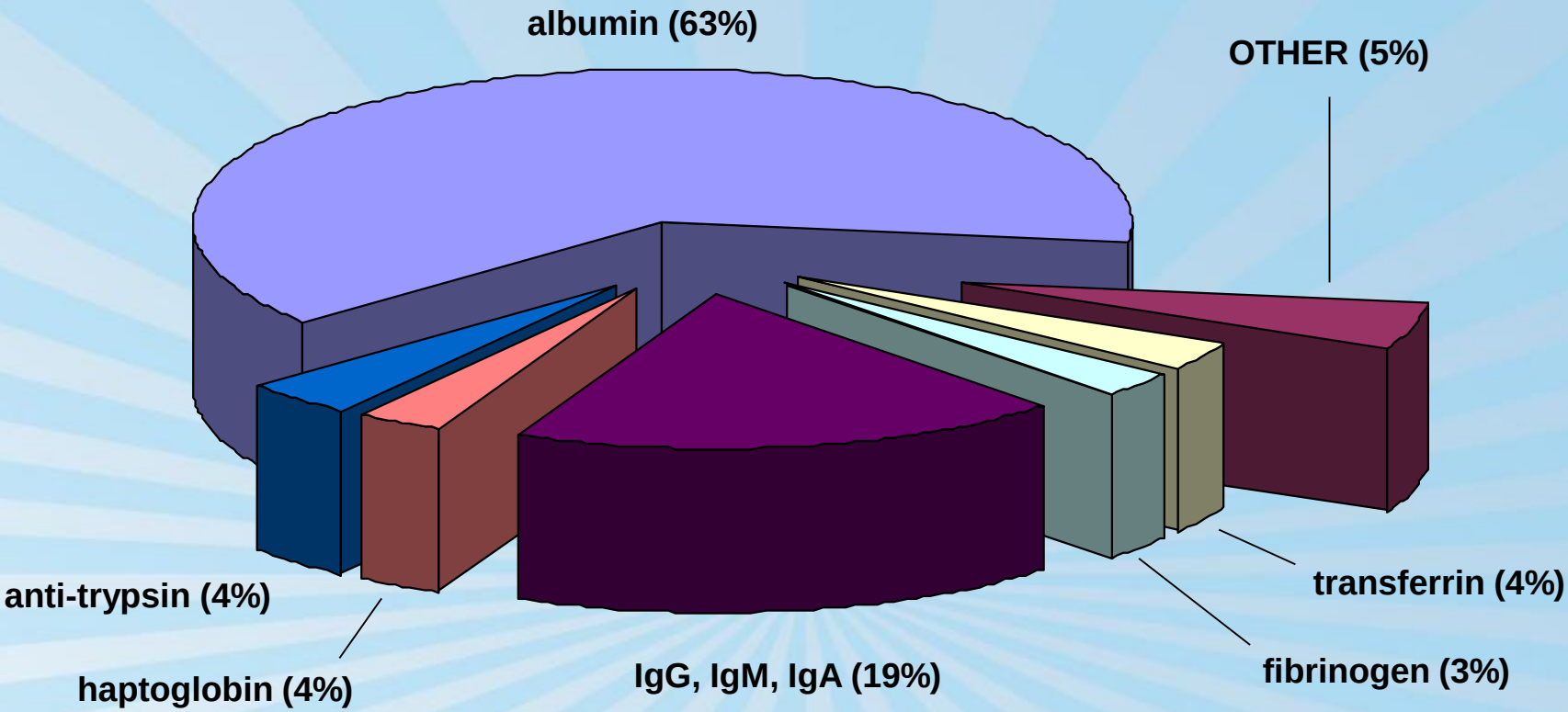

For Research use only!
Not for diagnostic or
therapeutic applications!
Catalogue-No. C2011
Batch-No. B 72 016 06 05

Six proteins constitute 95% of plasma proteins



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CDM-HD Serum Replacement

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Advantages of Hollow Fiber Cell Culture

- Concentrated product
- Uniform and complete post-translational modifications
- Low apoptosis, less contamination with intracellular proteins and DNA
- Protein free medium (CDM-HD) contains no surfactants
- Consistency of production over many months.

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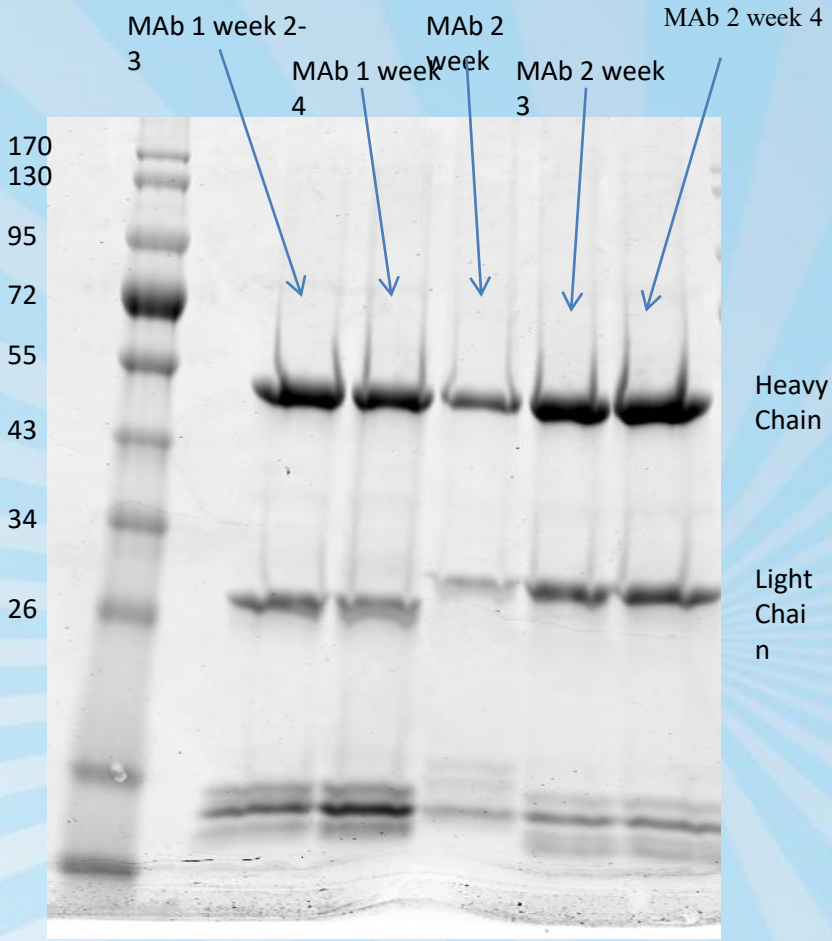
Monoclonal Antibody Production Using CDM-HD

Mab 1: 168 mgs in 60mls volume, 2.8 mgs/ml. 9 liters of medium consumed, three weeks culture

Mab 2: 159 mgs in 70mls volume, 2.3 mg/ml. 11 liters of medium consumed, three weeks culture



- TGF Beta diffuses out
- MAB trapped in ECS
- Easily adapt to SFM/CDM HD
- Lower endotoxin
- .5 to 5 mg/ml conc.
- 5-100 mg per harvest
- Continuous production for over 6 months

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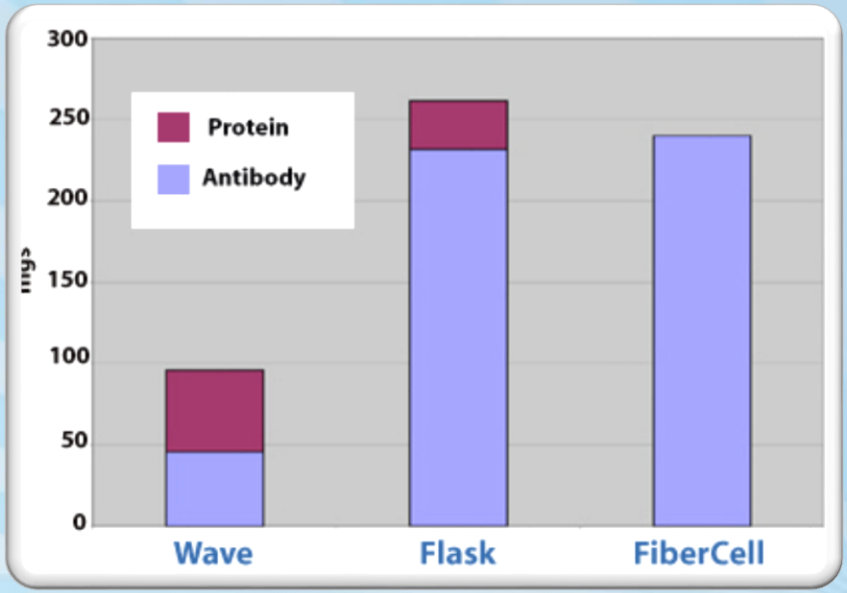
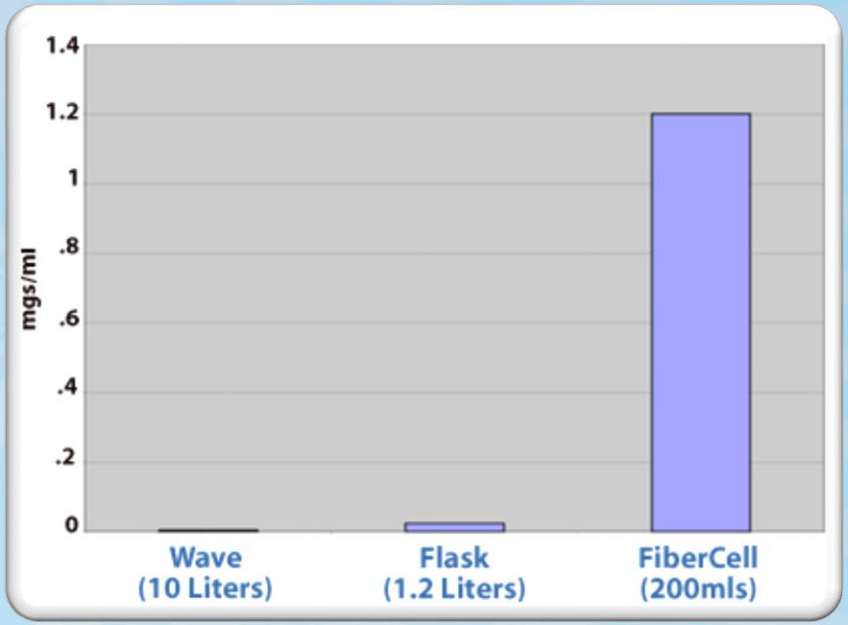
Hollow Fiber/Wave Comparison





Scale up of hollow fiber is limited by oxygenation

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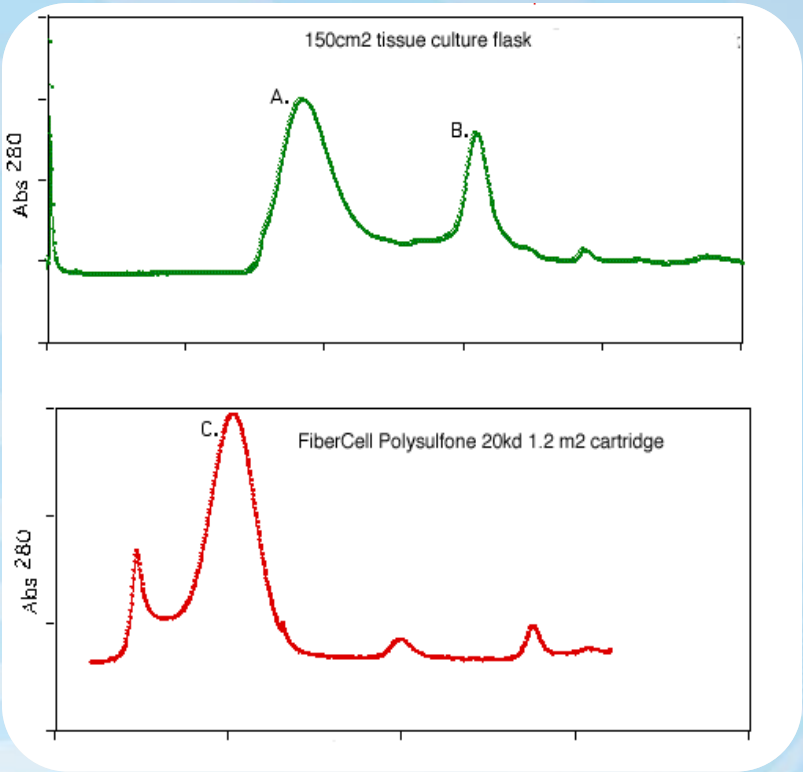
Recombinant Protein Production



- Both suspension and adherent cell types
- 100X + higher concentration
- Easily adapt to SFM
- Can provide improved protein folding

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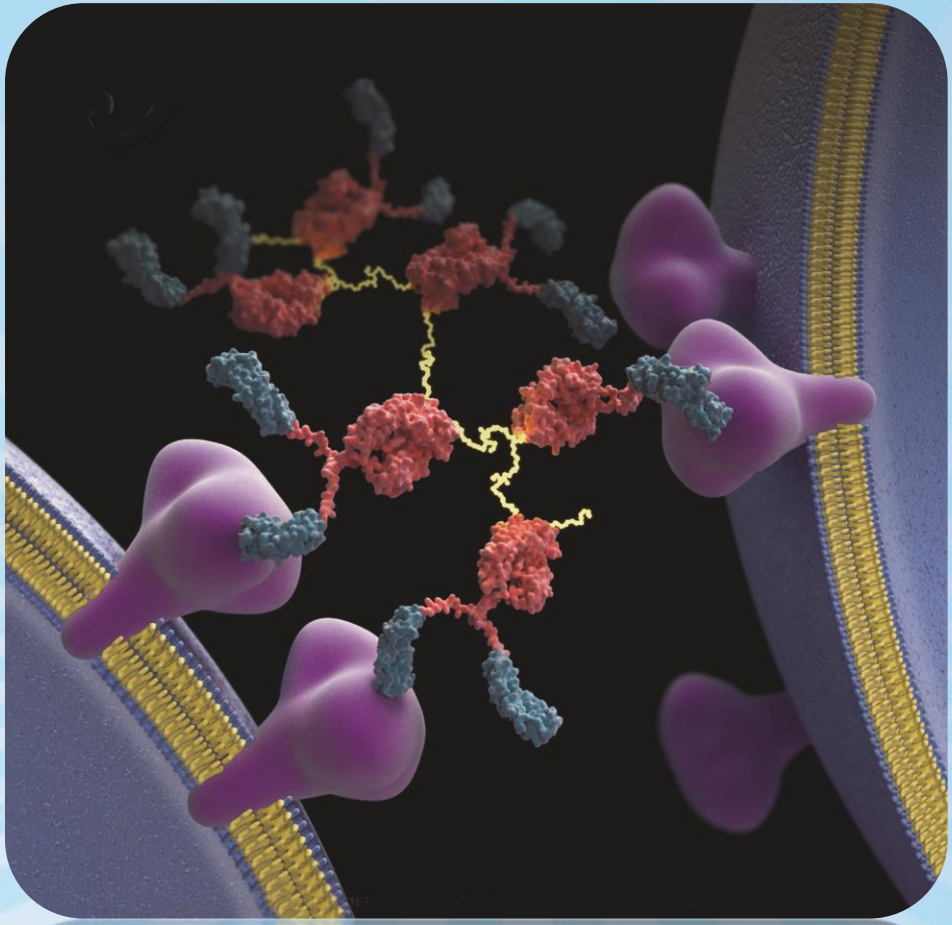
Journal of Biological Chemistry 9/20/2007



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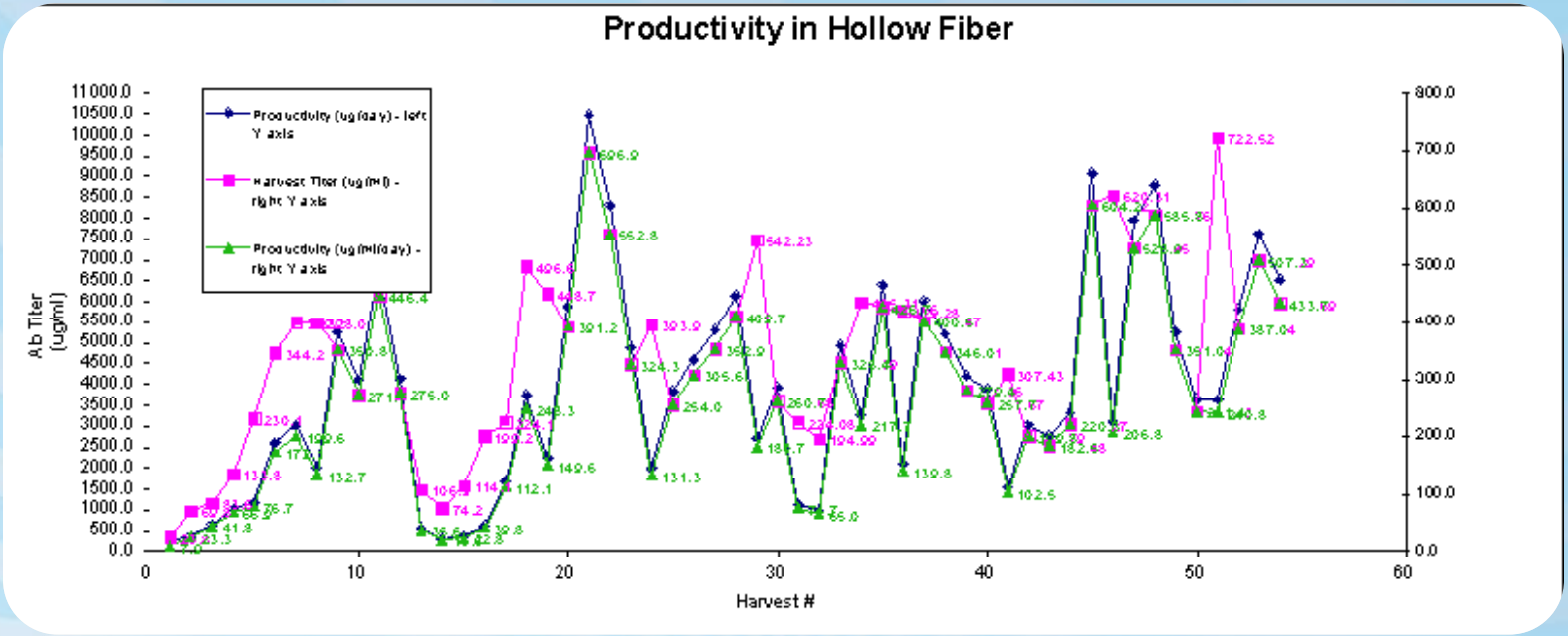
Rec. Protein Production in 293 cells



- Rec. IgG from 293 cells
- Produced 276 mg in 2 months using C2011
- 900 mL total volume in SFM

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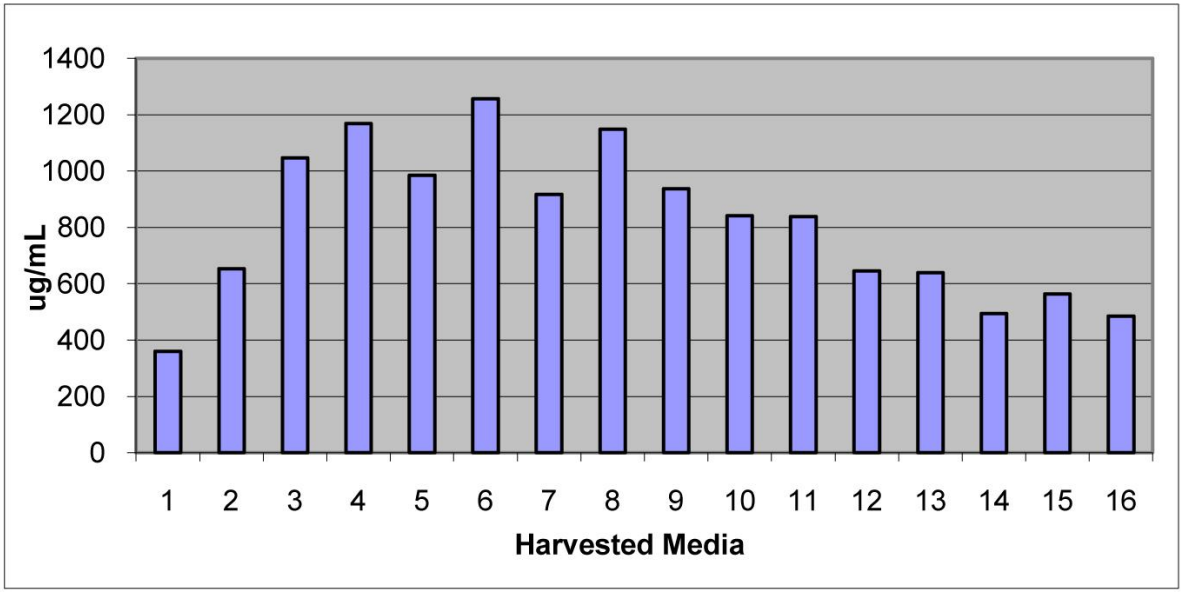
Cell Line CHO Suspension Cell (DG44)



- Protein produced: 246.6 mgs
- Harvest Volume: 304 mls
- Medium consumed: 10 liters
- Culture time: 35 days

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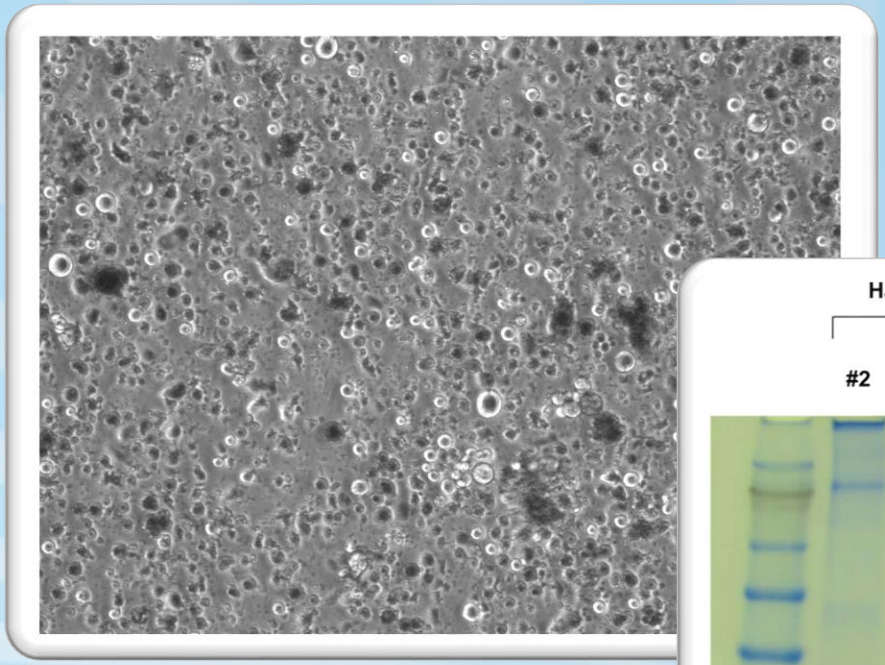
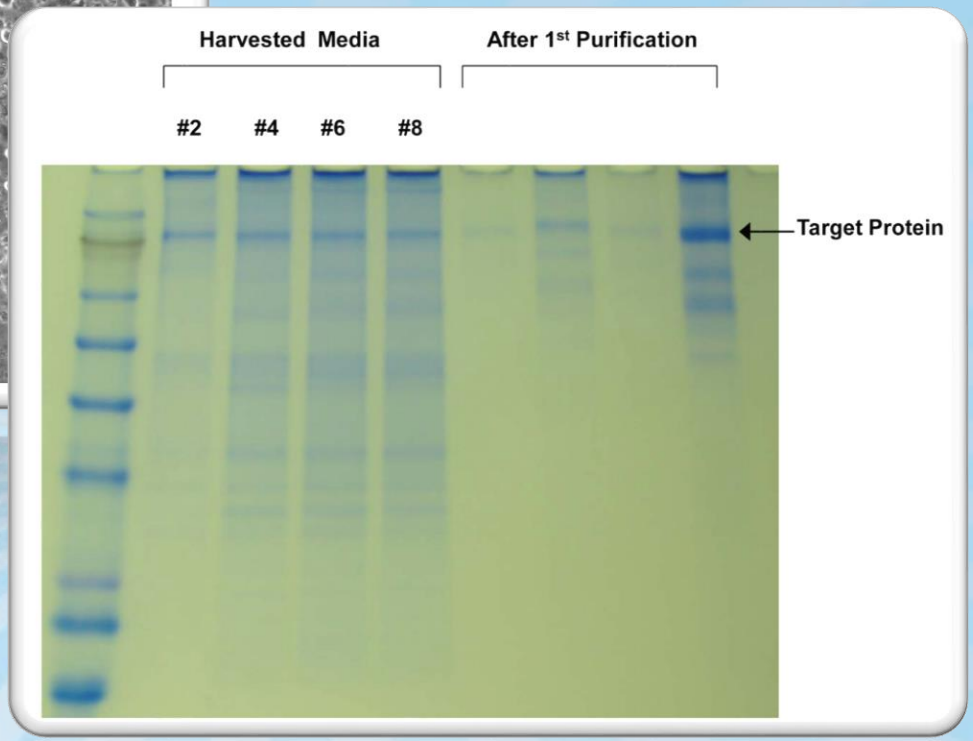
Raw Harvests from DG44 CHO Cell Line





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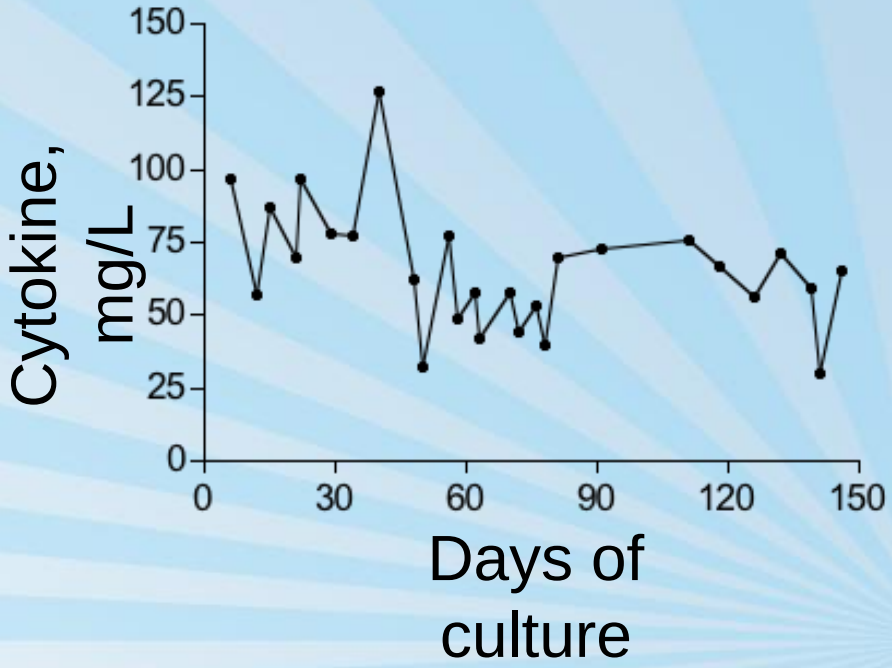
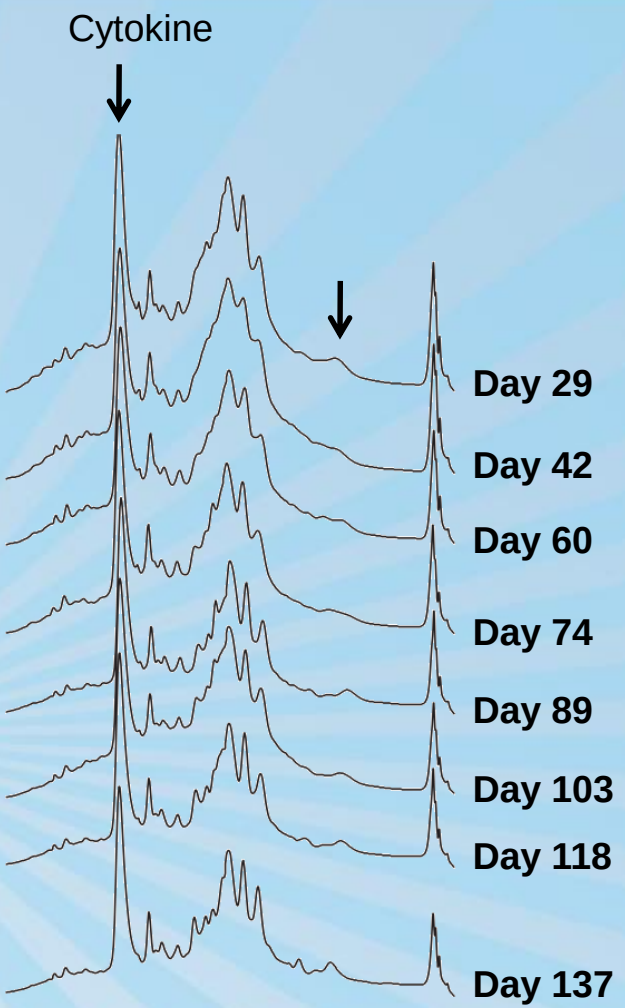
Stable Cytokine Production In Fibercell Hollow Fiber Bioreactor Over 5 Months



Fibercell Production, mg/Lt



RP-HPLC Analysis, Serum Free Harvest

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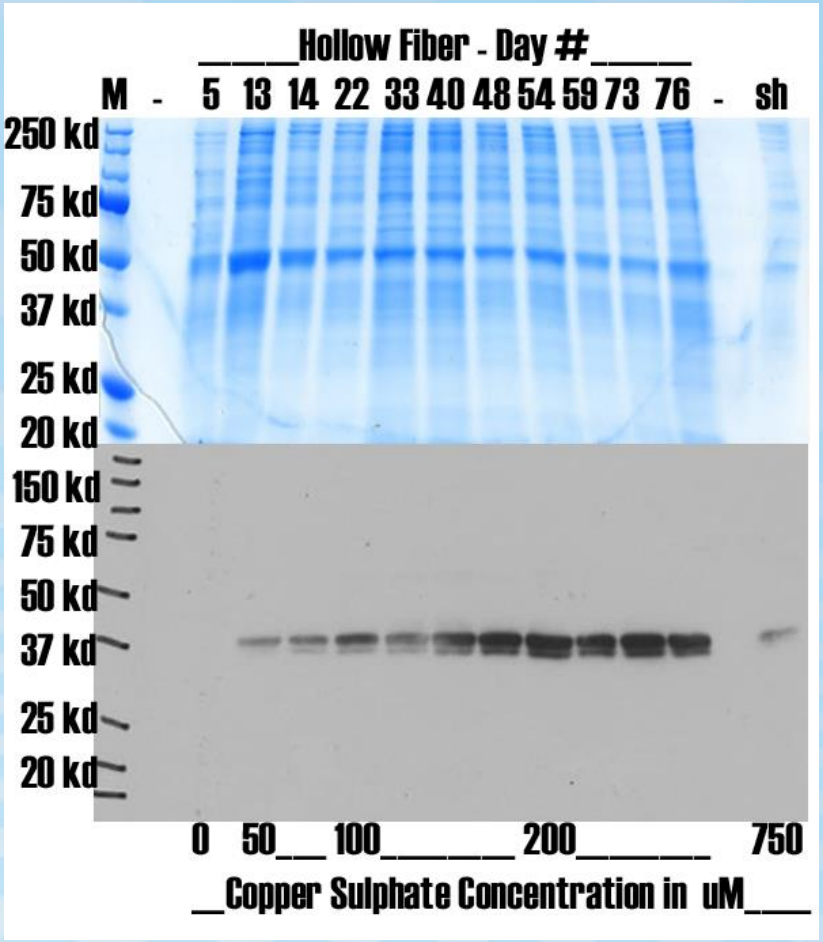
Bergamaschi & Pavlakis, NCI

S2 Protein Production



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FiberCell Equivalents

- A single harvest from C2011 can equal 20 roller bottles per day
- A single harvest from C2018 can equal 200 roller bottles per day
- 10mL harvest volume can equal 1 liter of cell culture supernatent

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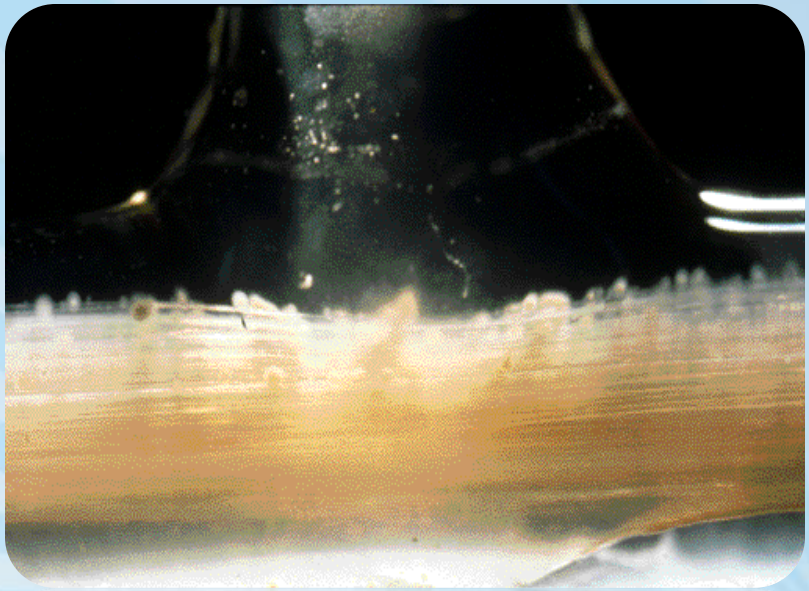
Cellular Co-cultivation



- Only way to get two cell types in close enough proximity and high enough density to observe effects
- Thymic epithelium and thymic fibroblasts co-cultured to form thymic nodules

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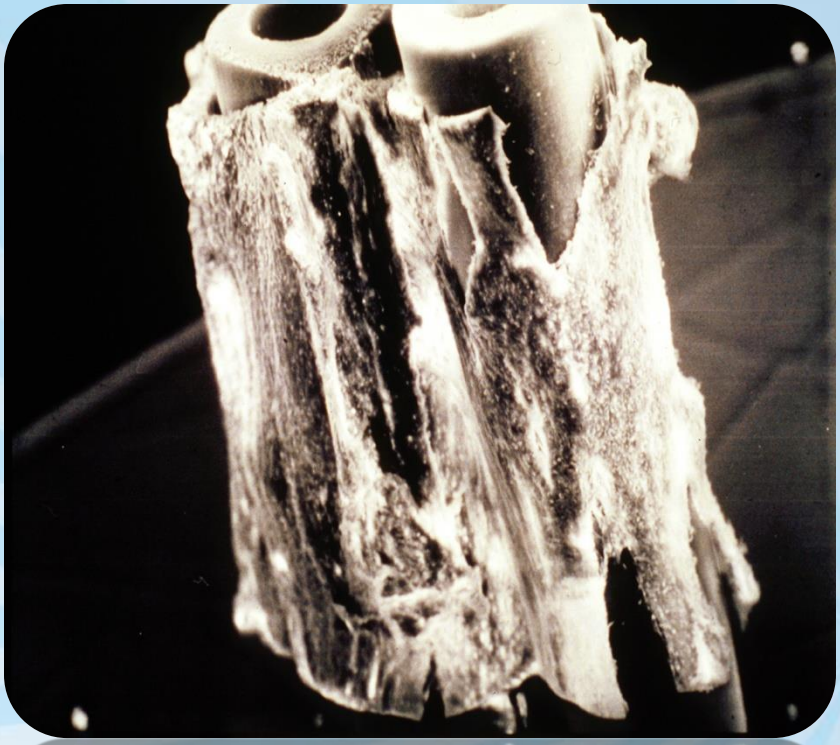
Asymmetric Cell Co-cultivation



- Co-cultivation of endothelial cells (inside) and vascular smooth muscle (outside)
- Brain endothelial and astroglial cells to form in vitro blood brain barrier

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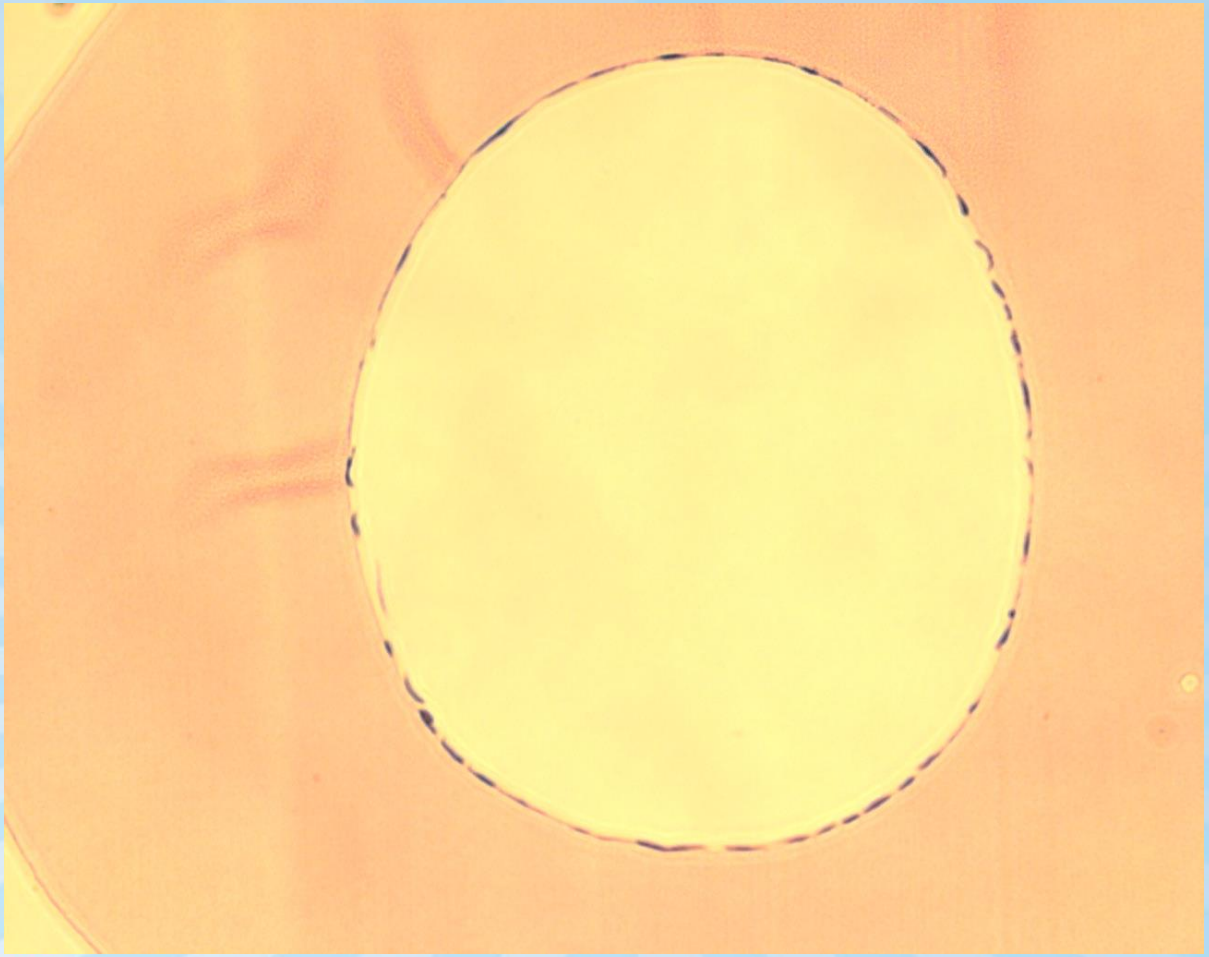
Endothelial Cell Culture



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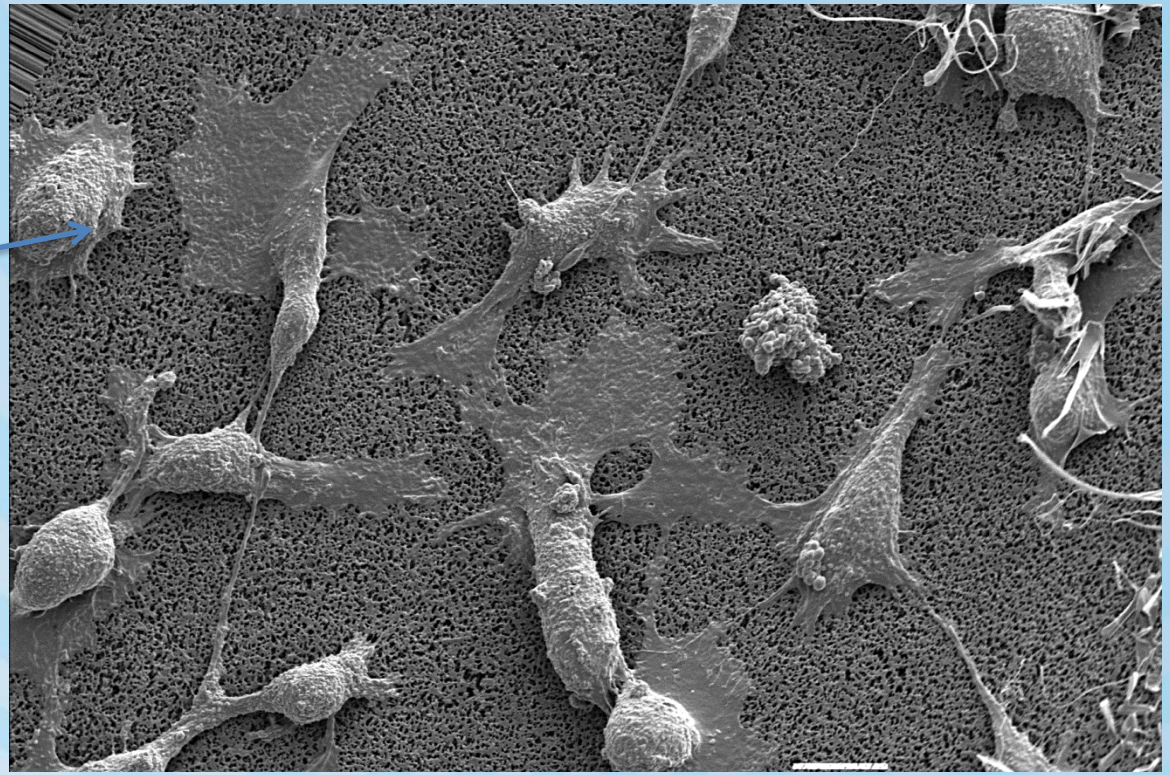
Pores of fiber



Endothelial cells on the inside on a fiber- these have been adhered to the wall then subjected to very low shear force overnight followed by a few hours at 5 dynes/cm^2.

While the majority of the cells here are still bulky it is possible to see then begin to flatted down onto the wall of the fiber and really stretch out.

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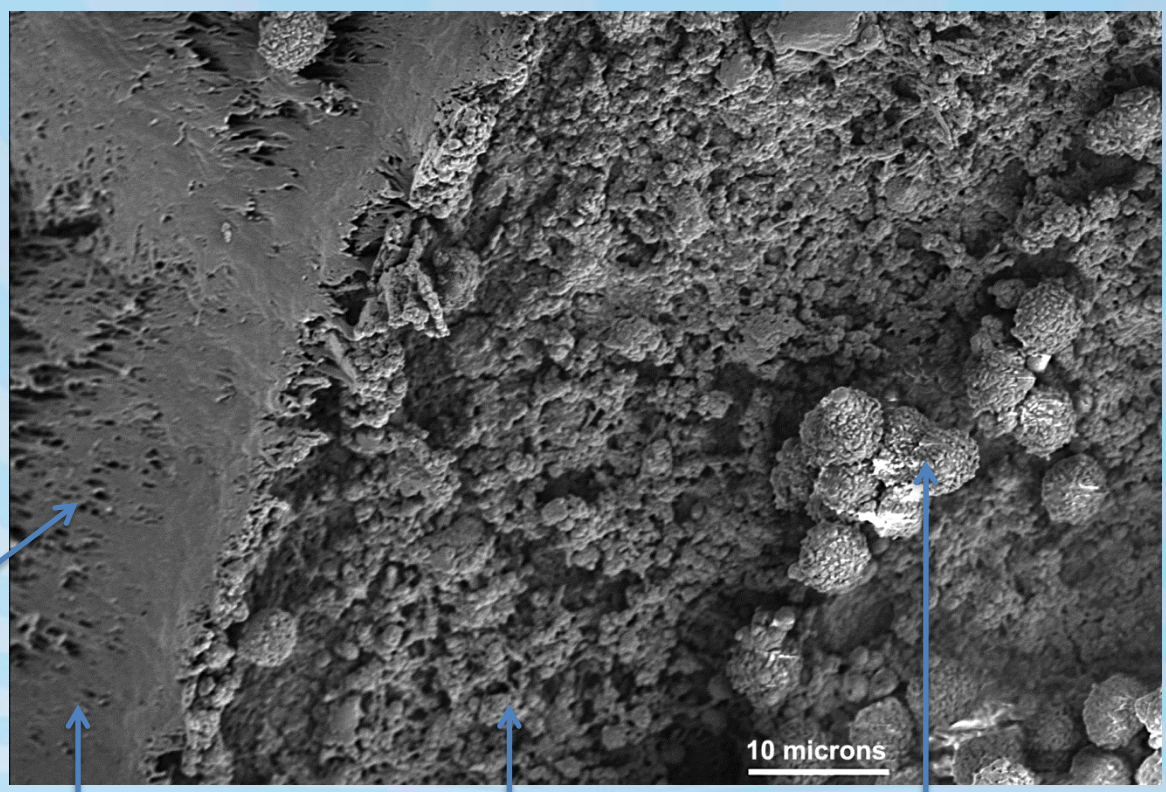

Pores

Fiber

Inside of fiber

CLL cells

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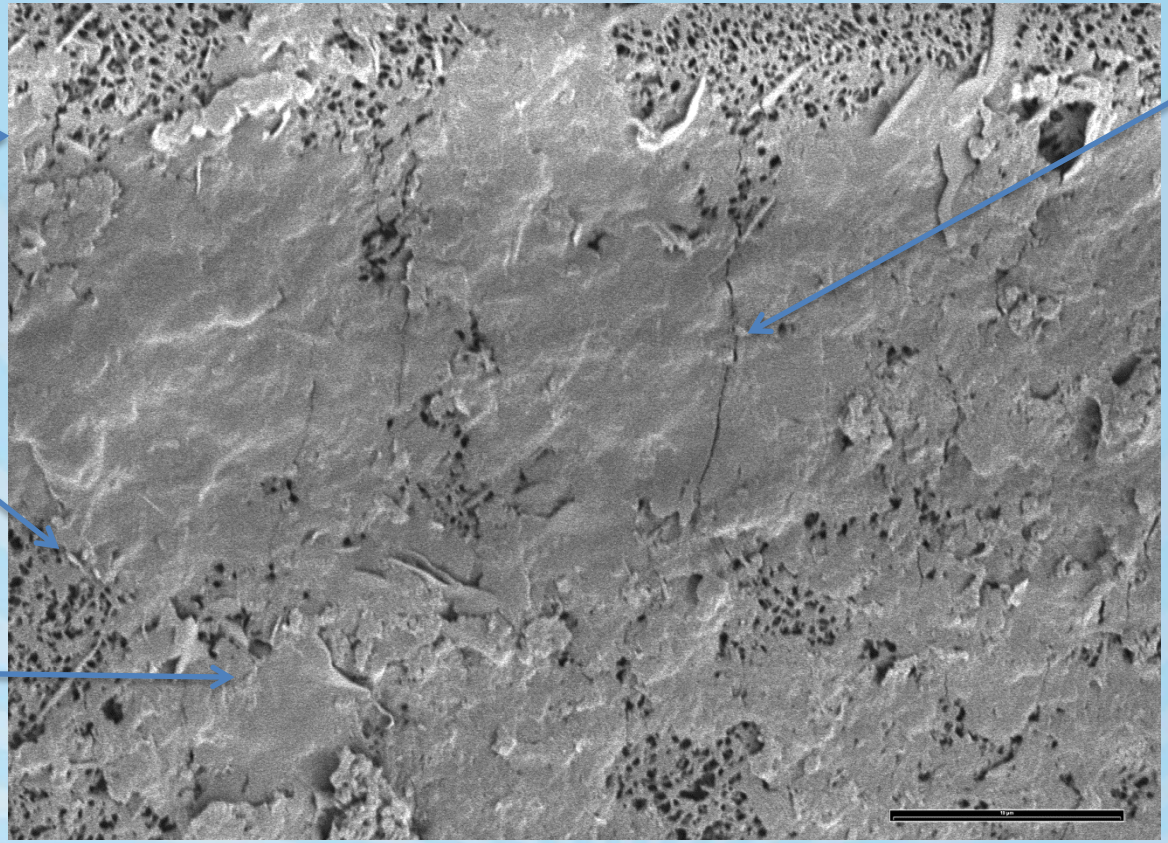
Inside of hollow fiber

Pore

Endothelial cell



Crack! Likely to be a result of trying to mount a sliced hollow fibre during microscopy

Flattened endothelial cells on inside of fiber. These endothelial cells had been adhered to the inside of the fiber and subjected to minimal shear force over night followed by a minimum of 5 hours at 10 dynes/cm2. This was based on results we saw when testing the application of a different system that can apply shear force to channels lined with endothelial cells while visible under a microscope. This other system also allowed us to discover that endothelial cells will detach and roll away after alignment if a bubble passes over them so we eliminate all bubbles from the system to preserve the endothelial layer.

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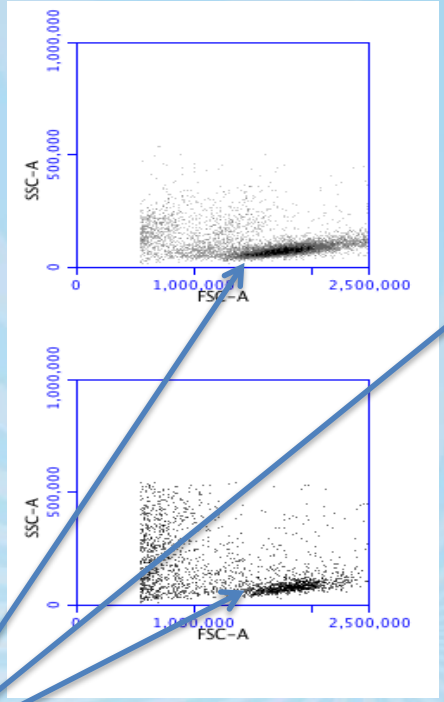
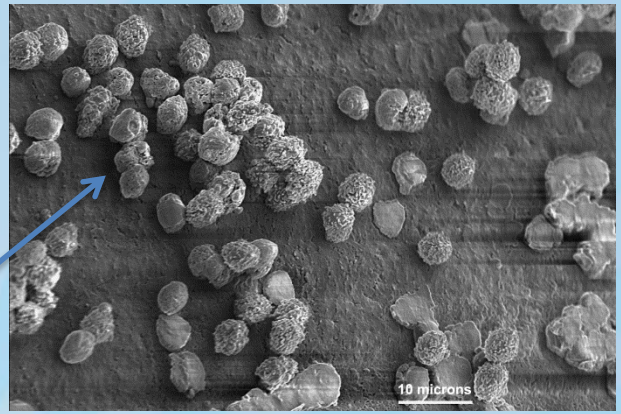
CLL cells actively migrate into the extra-vascular space

Circulating Compartment

Extra-vascular space (migrated compartment)

CLL cells





Scanning electron micrograph of the outside of a hollow fibre after circulation of CLL cells around the system

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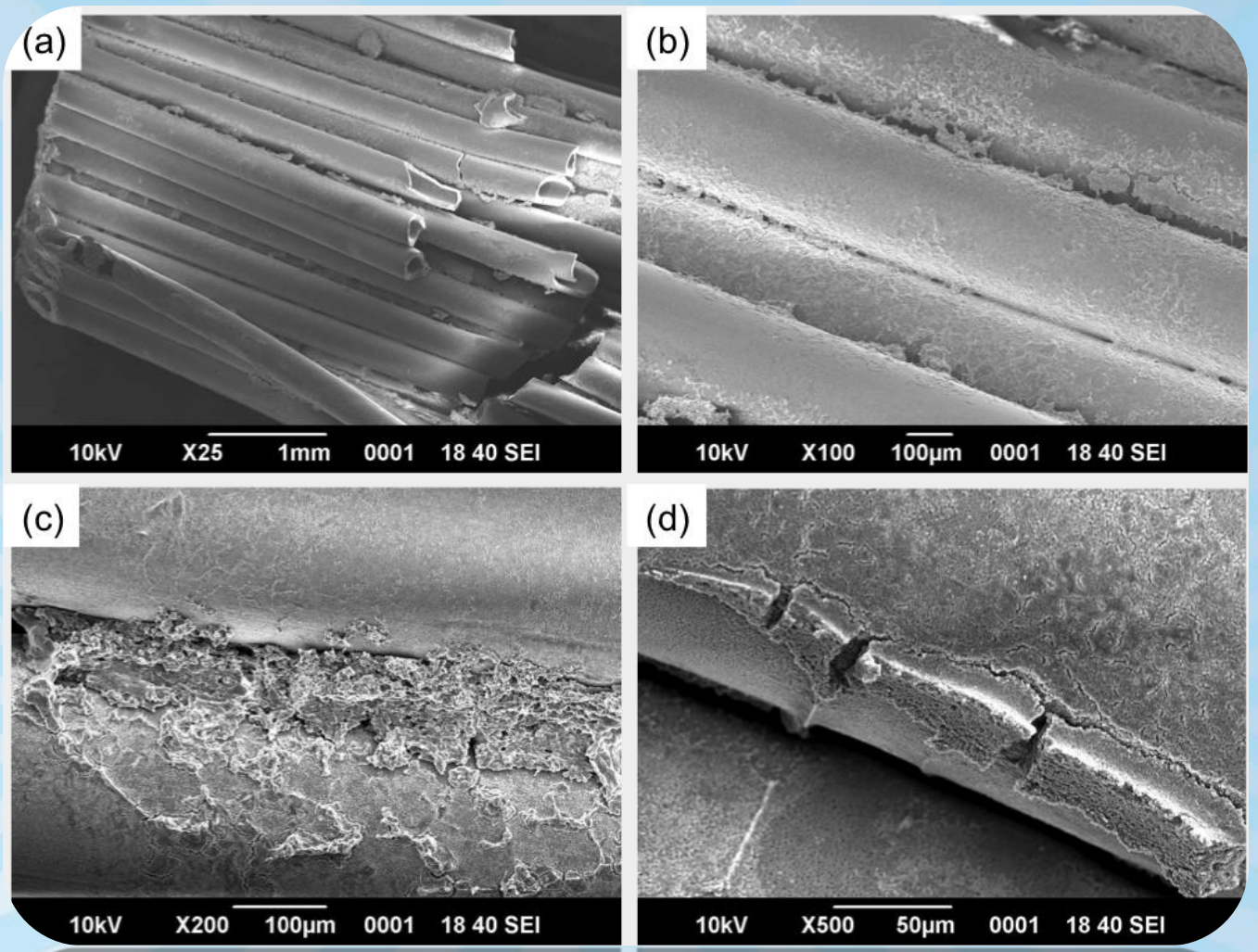
Bone Marrow Stroma/HSC Co-Culture



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Lack of new antibiotics

- Only 2 systemic antibiotic agents approved since 2008
- 16 approved between 1983 and 1987
- 3 reasons:
  - **Scientific:** Easy to discover antibiotics have already been found
  - **Economic:** Antibiotics represent a poor return on investment and new antibiotics reserved for difficult cases
  - **Regulatory:** FDA approval process increasingly complex and expensive.

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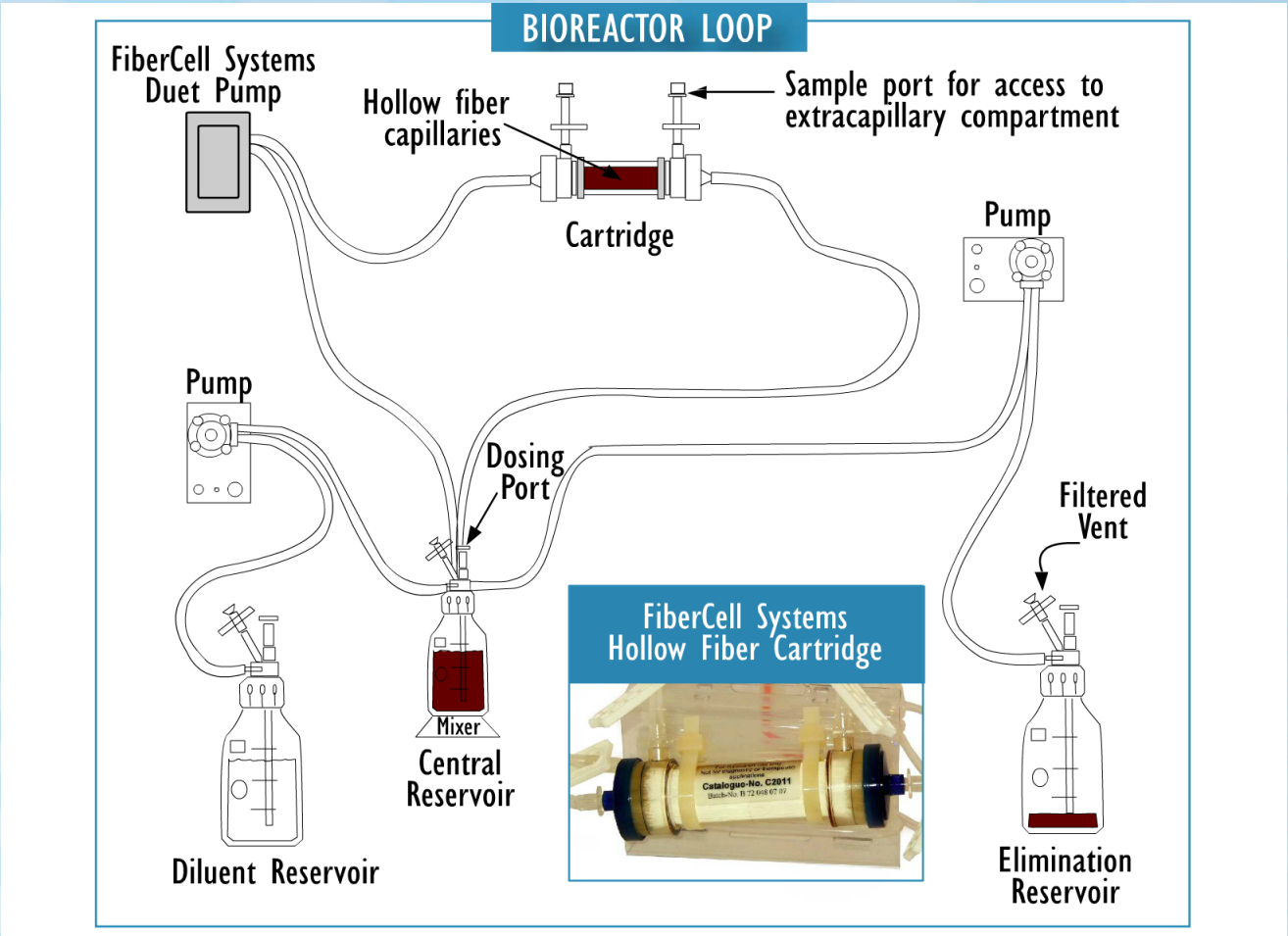
Hollow Fiber Infection Model



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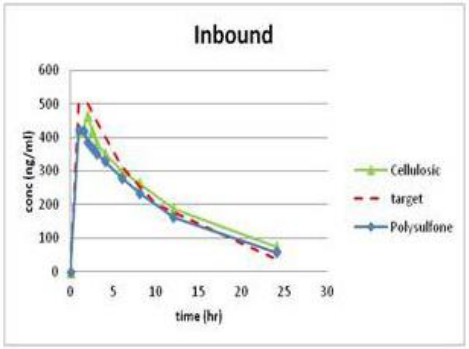
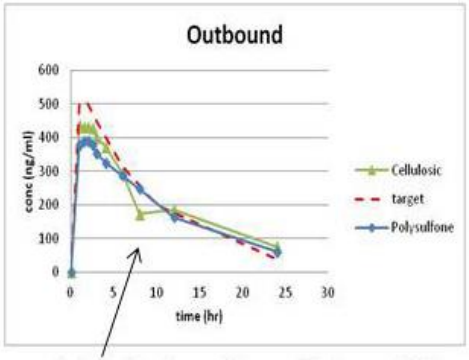
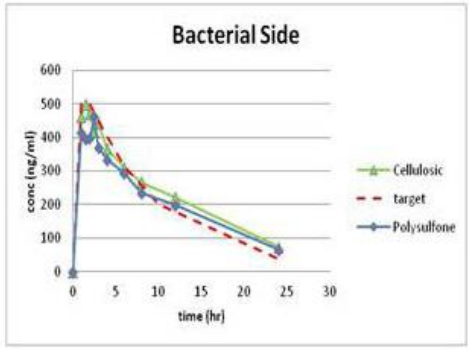
PK Profile

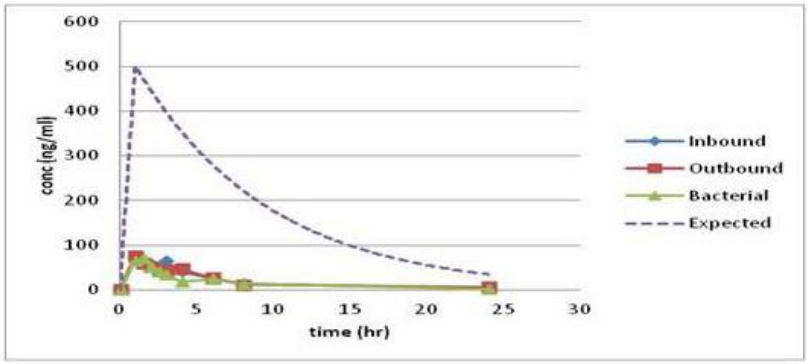
This drug showed good compatibility with both cartridges.







Not significant enough to consider incompatibility



This drug is not a good candidate for HFS use

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The hollow fiber infection model is a complementary and additional tool for drug development, to be implemented at the earliest stages

- Optimal dose selection and route of administration
- Optimal dosing schedule
- Possible combination therapies
- Defines emerging resistance
- Defines total kill
- Post-approval drug regimen optimization
- Can support trial design for Phase I, II, III and IV clinical trials

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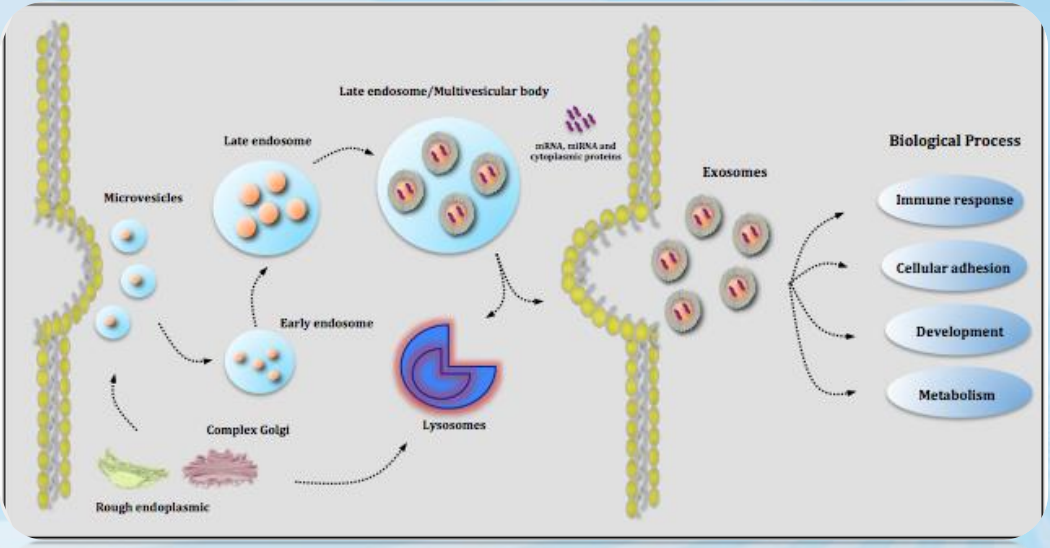
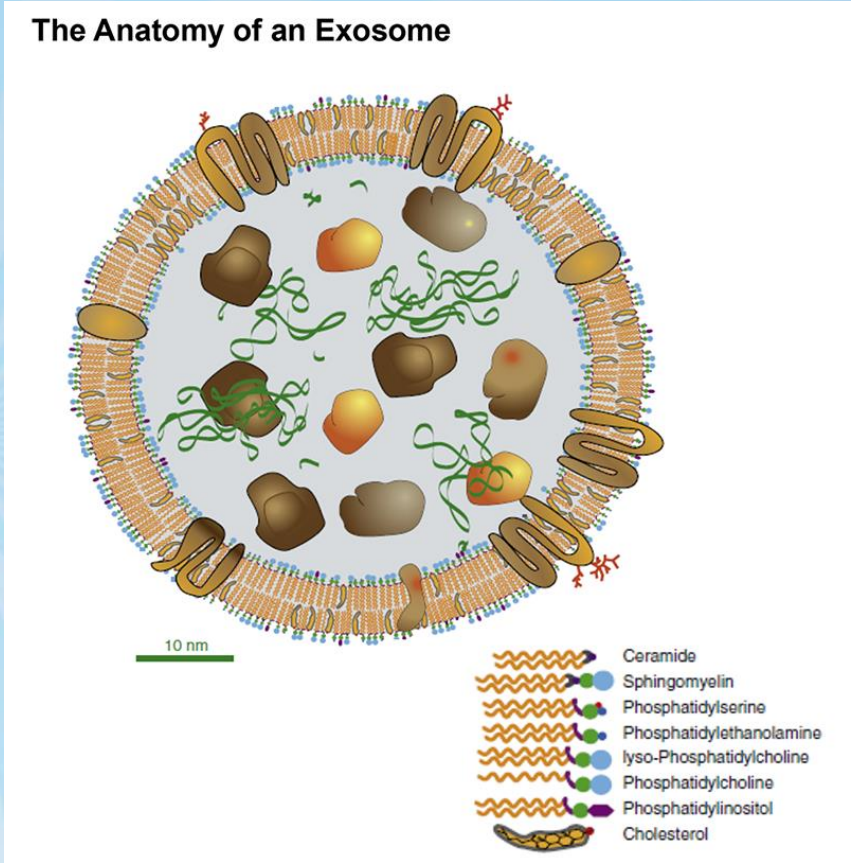
Exosomes

- Cell-derived vesicles in biological fluids
- Including medium of cultured cells
- Dia. between 30 and 100 nm
- Contain cellular proteins and RNA
- Facilitate cell-to-cell transfer of cargo
- May play a role in cell-to-cell signaling
- May mediate adaptive immune responses





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Human Adipose Derived Adult MSC

130 T225 Flasks

- Harvest Volume: 4000mls
- Protein: 0.9 mgs
- Particles: $1.6X10^{9}$

One C2011 Bioreactor

- Harvest Volume: 120mls
- Protein: 14.45 mgs
- Particles: $3.27X10^{12}$

$5X10^{8}$ cells seeded

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Culture Platform (1×10^9 cells)	Medium Volume (mL)	Total Number of EVs (10^9)
Bioreactor Harvest #1	40	320
Bioreactor Harvest #2	40	250
Bioreactor Harvest #3	40	290
130 Flasks (225 cm^2)	4000	16

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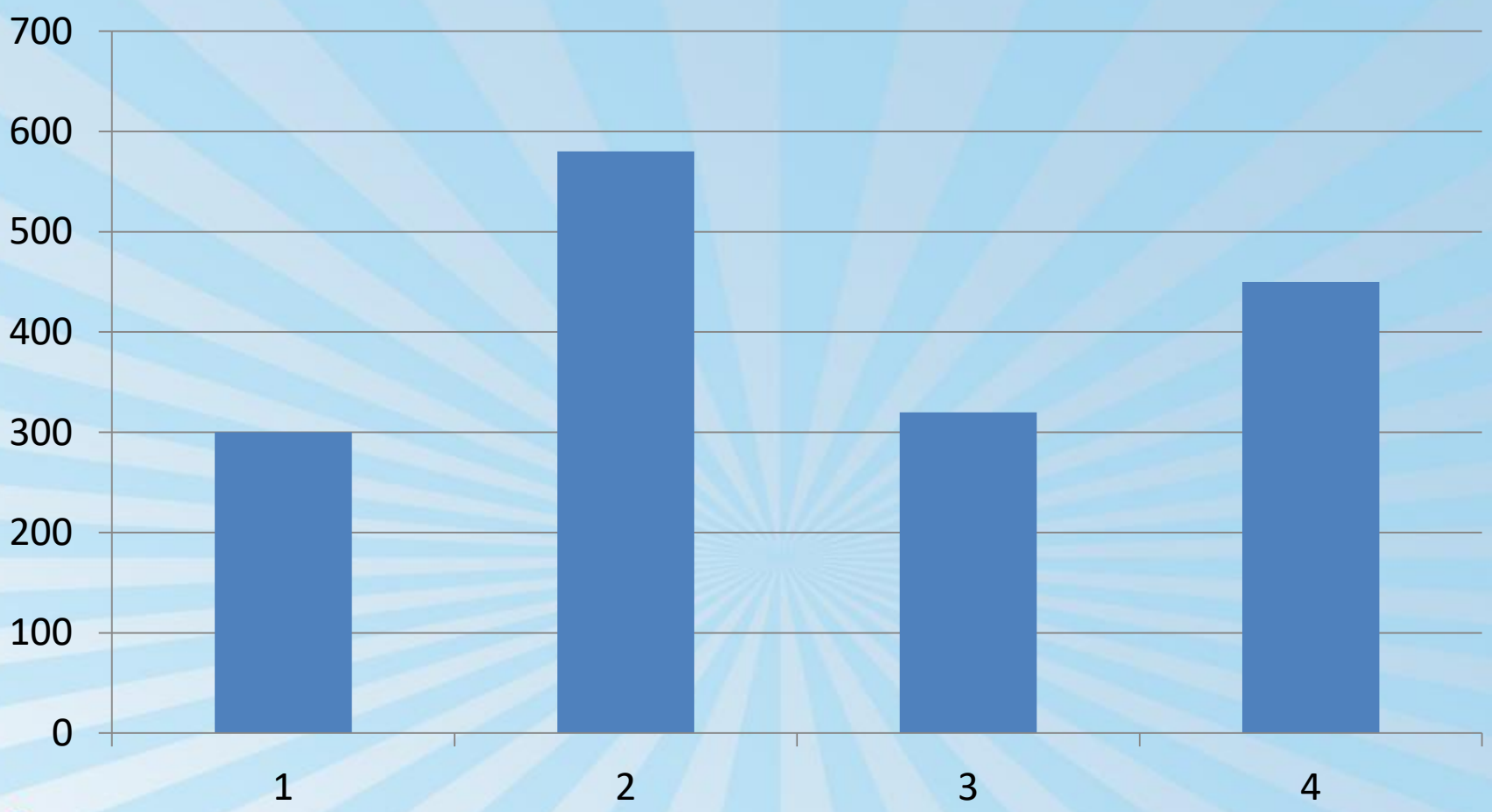

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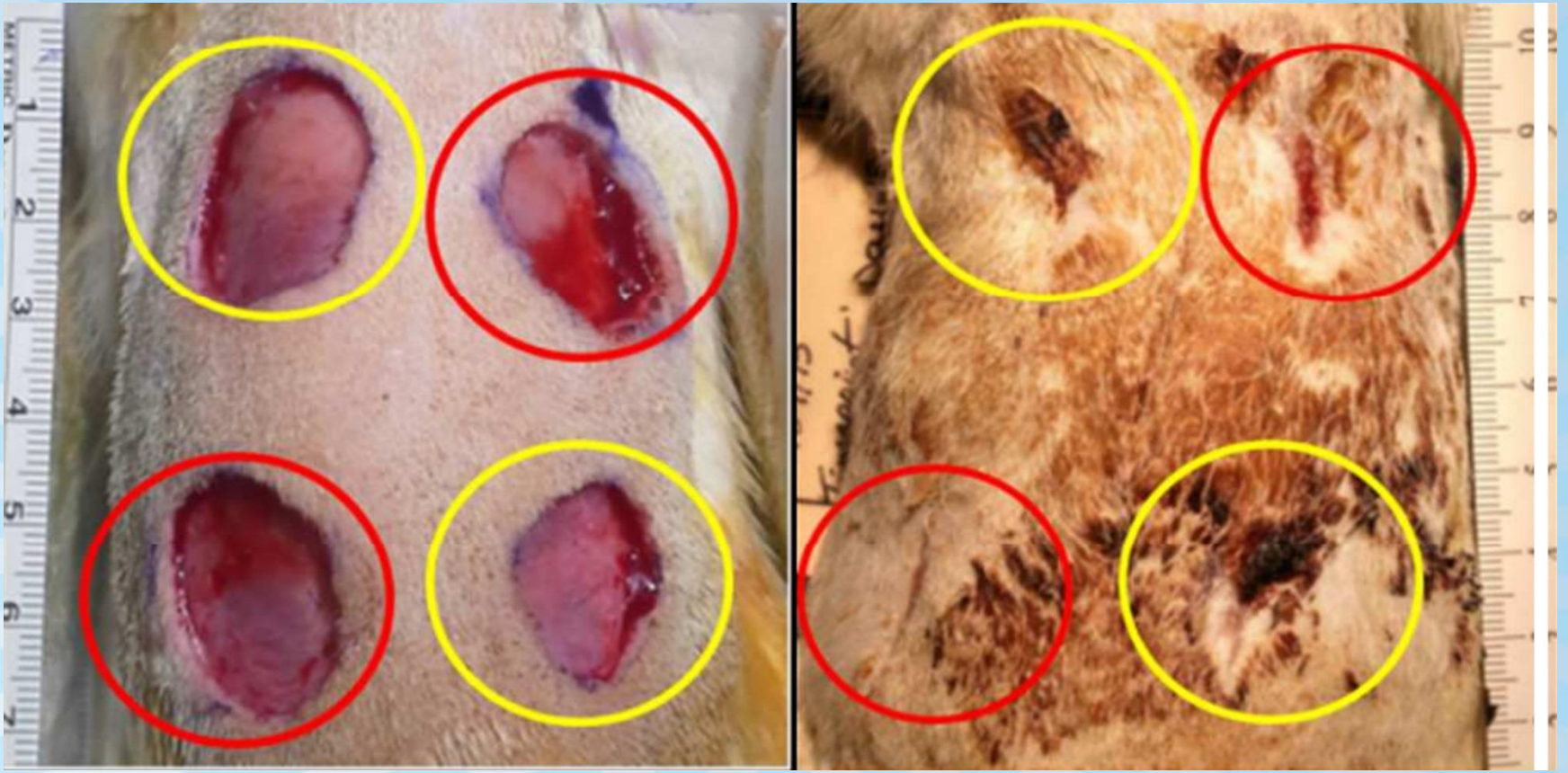

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Advantages for Exosome Production

- Large numbers of cells can be cultured in a small space
- Secreted exosomes are concentrated
- Continuous production over several months
- Serum can be used without contamination from endogenous exosomes
- CDM HD can be used for cGMP production
- Cell proliferation may be limited

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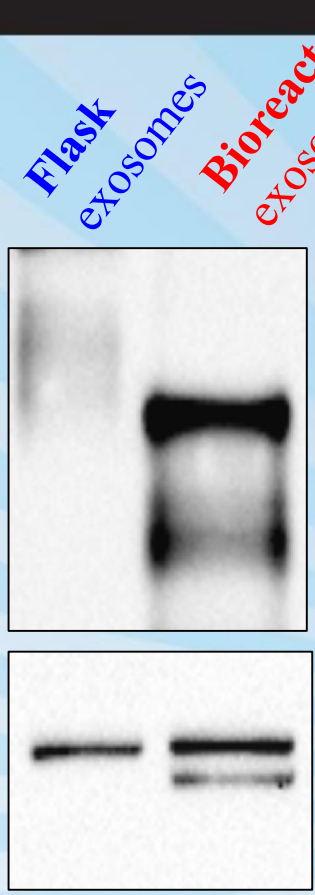
Bioreactor is a rich source of exosomes



Sample	µg yield per ml sup
Conventional culture EVs (comparison protocol)	**2.7 ± 0.4**
Bioreactor EVs (comparison protocol)	**33 ± 3**
Bioreactor EVs (optimized protocol)	**54 ± 2**

>10-fold increase in exosome yield from bioreactor supernatants

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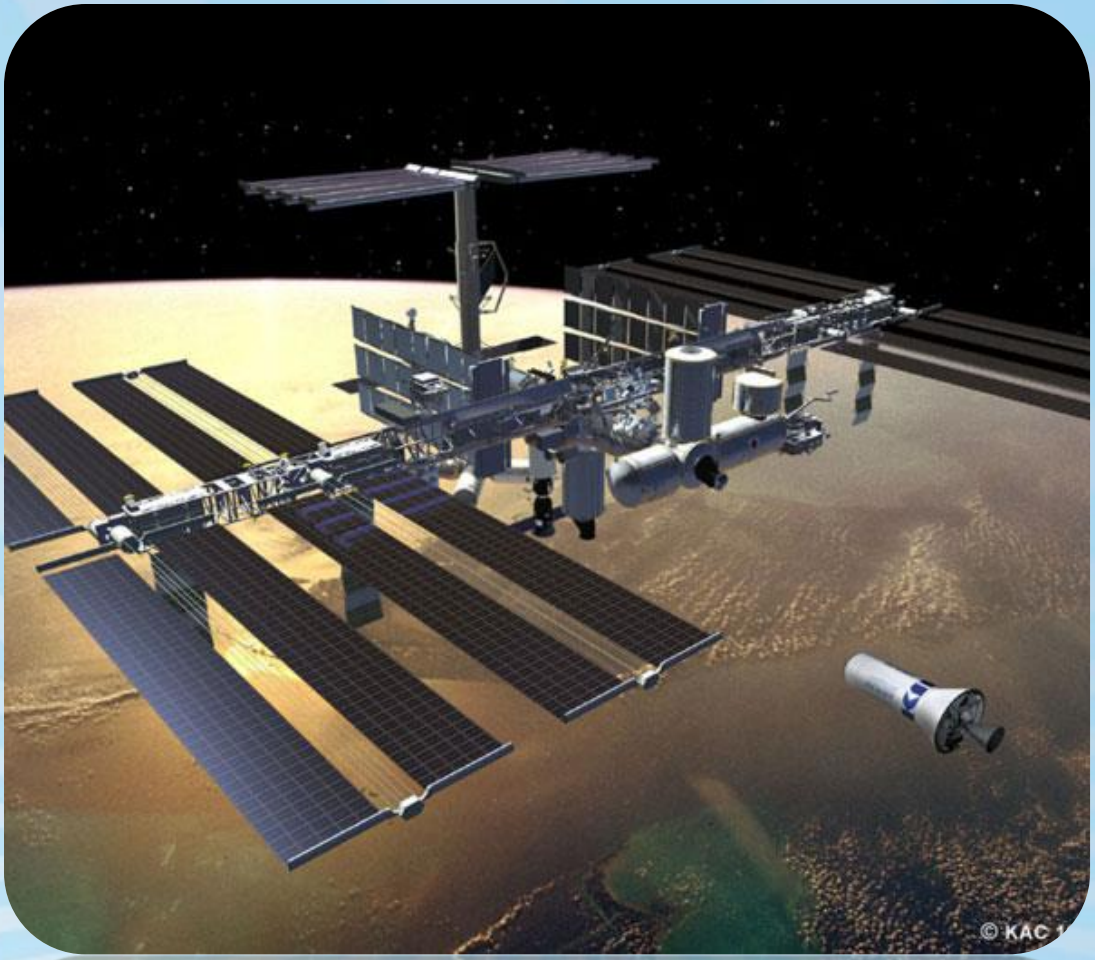
Dionysios C. Watson, Jenifer Bear, George N. Pavlakis
National Cancer Institute, USA



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Pulsatile Perfusion of Placenta

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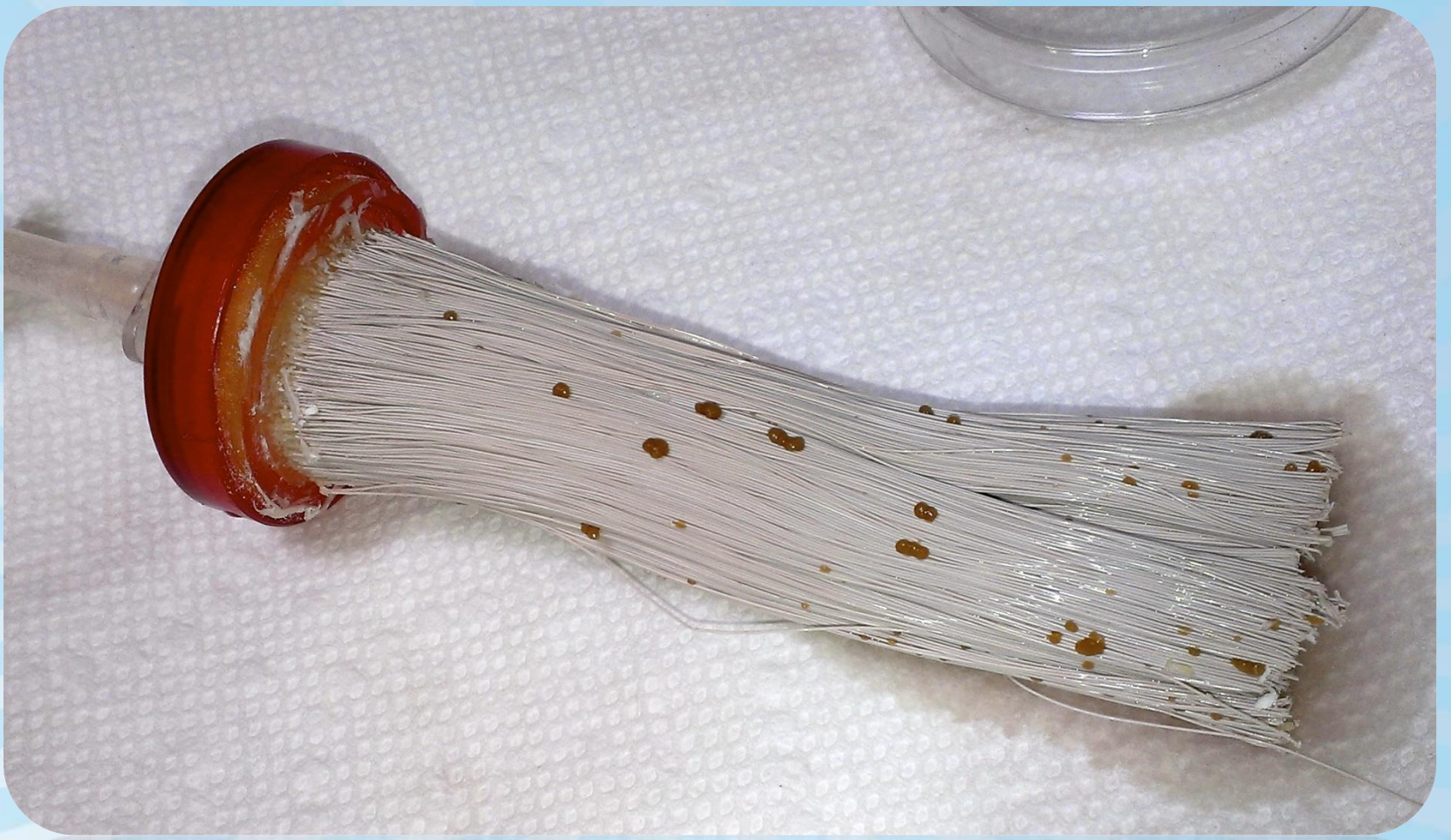
Placental Co-Culture



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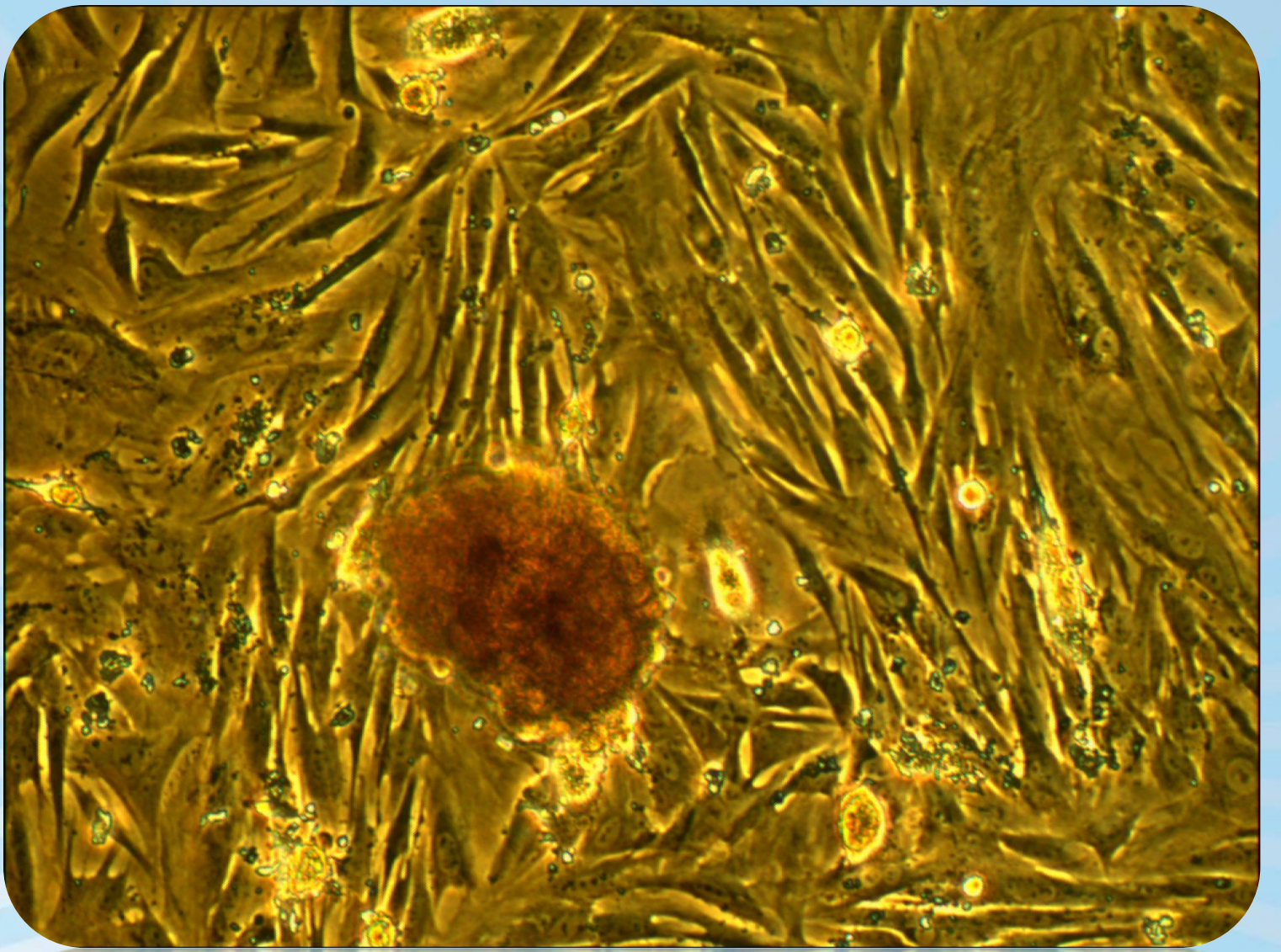

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Harvest vs. Flask

Phenotype	ECS Harvest	Flask
• CD 45	4%	1%
• CD 34	0%	0%
• CD133/2	2%	0%
• CD31	3%	48%
• CD 13	6%	83%
• CD 105	43%	99%
• CD 73	18%	99%
• CD 90	5%	96%
• CD 14	23%	4%
• NANOG	0%	0%

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Summary

- Hollow fiber bioreactors are the method of choice for the culture of 10^9 to 10^{11} cells
- Ideal for producing 100mgs to several grams of MAB, 10mgs to 100s of mgs of recombinant proteins
- Concentration of products 10 to 100X higher than with conventional methods
- The most *in vivo* method for culturing cells over long periods of time
- Saves time, space, purification costs

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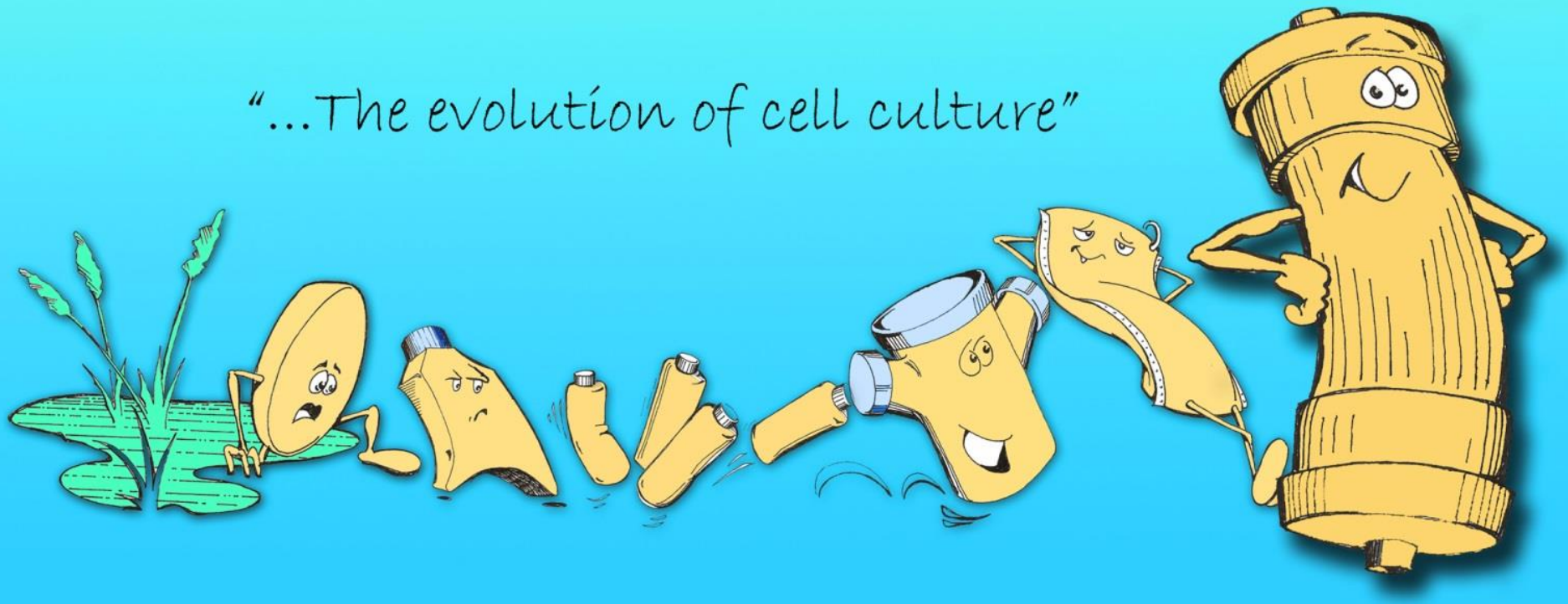
"...The evolution of cell culture"



Thank you.

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Recombinant Protein and Monoclonal Antibiotic Production in Hollow Fiber Bioreactors

Lab Scale To Process Scale

By John J.S. Cadwell

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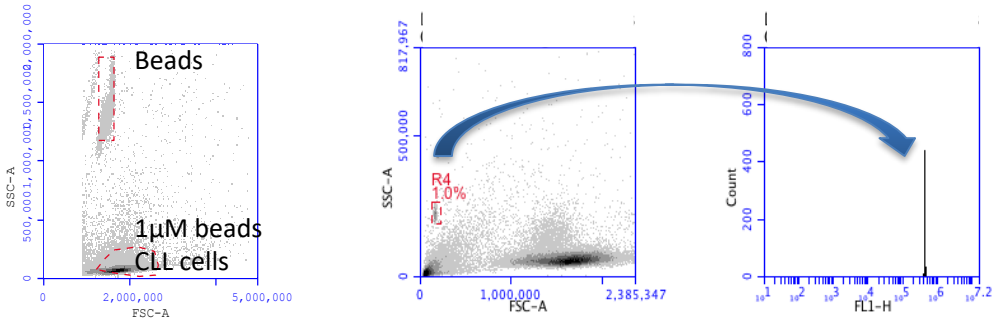
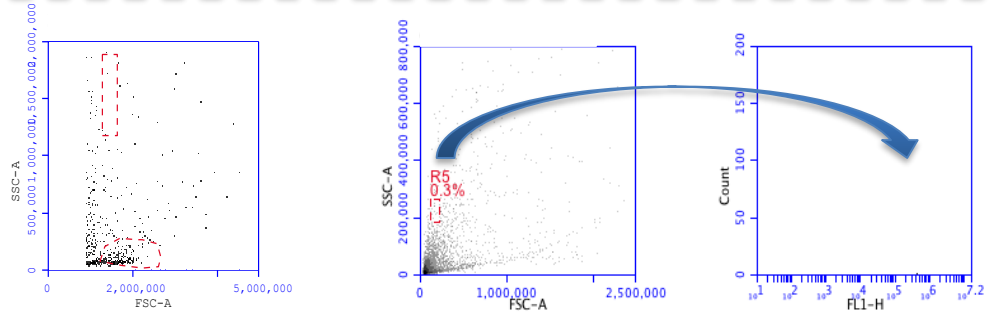
CLL cells actively migrate into the extra-vascular space

Circulating Compartment



Extra-vascular space (migrated compartment)



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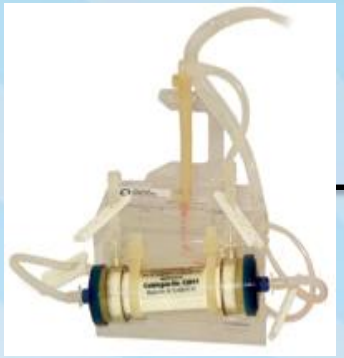
Exosome isolation via ultracentrufugation



60ml (3 ea.) Fibercell harvests, cleared of cells

Centrifuge sup @: **3,000xg** (15min) to remove cells and debris

Centrifuge sup @: **20,000xg** (30min) to remove large vesicles

Filter through 0.22µm

100kDa MWCO for ~10x concentration

a) Pellet exosomes by 110,000xg ultracentrifugation for 2h
b) Wash by resuspending in 6ml PBS, and repelleting @120,000xg for 90min

Resuspend in 1ml sucrose buffer*

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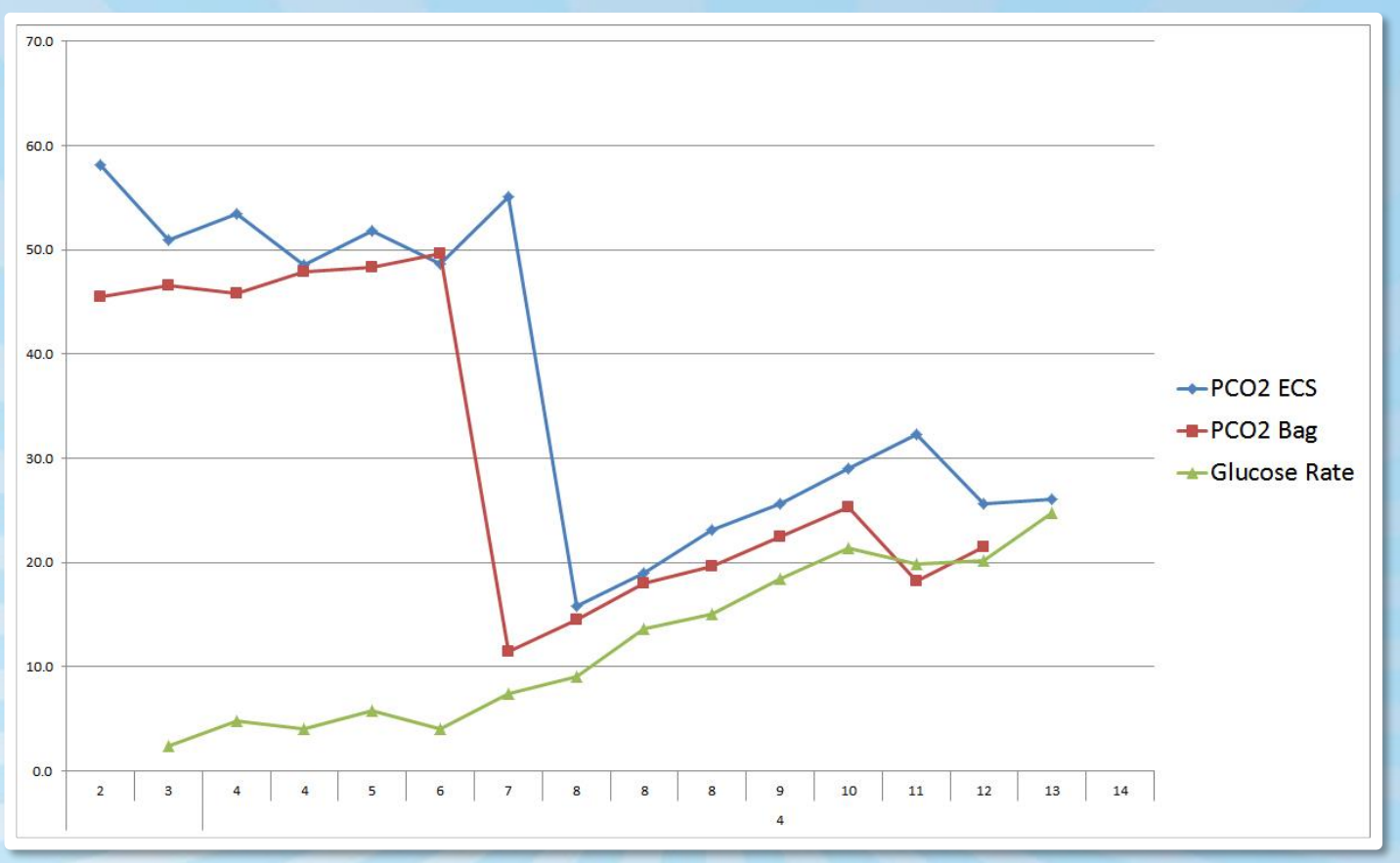
Data showing exchange across the fiber



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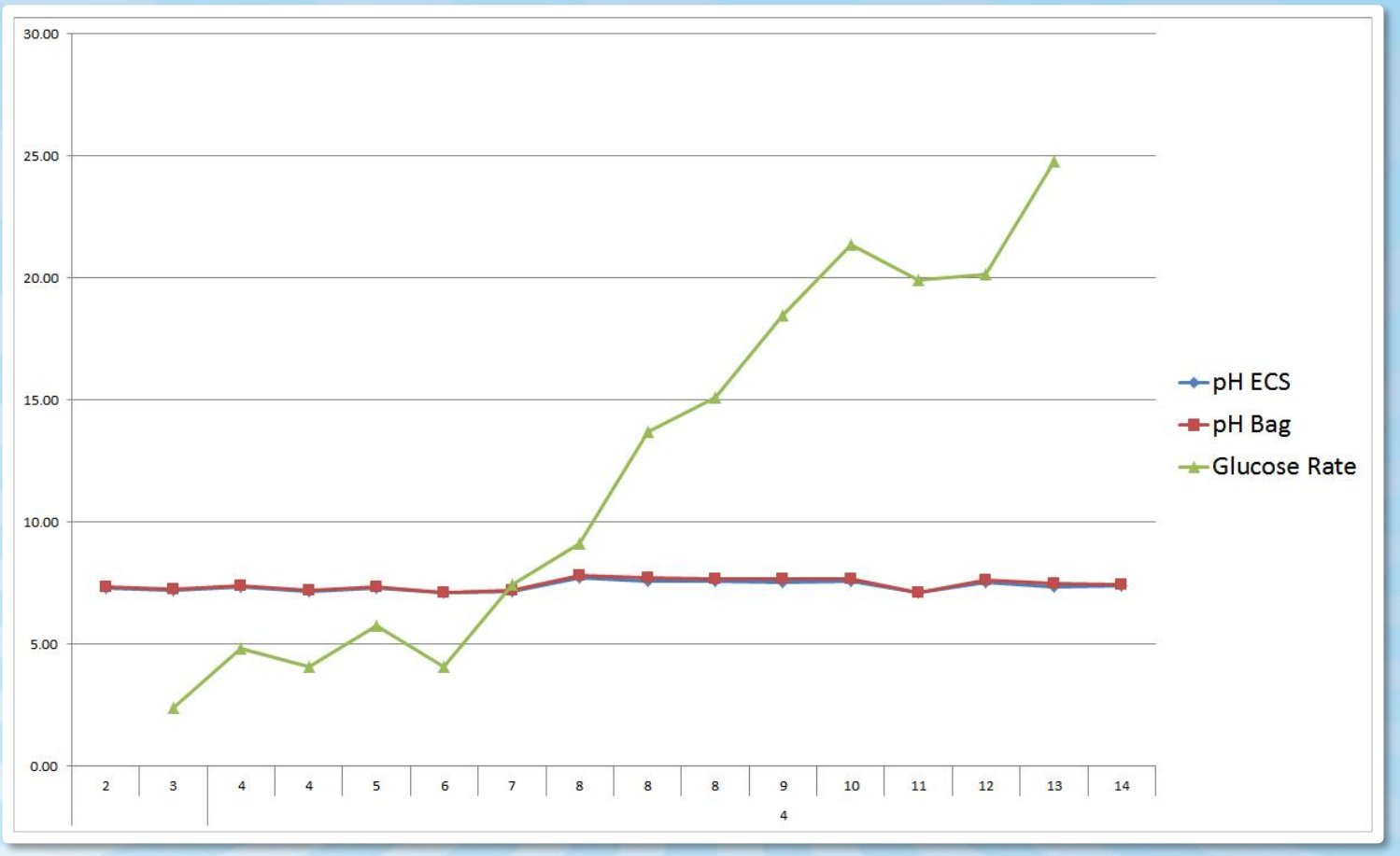

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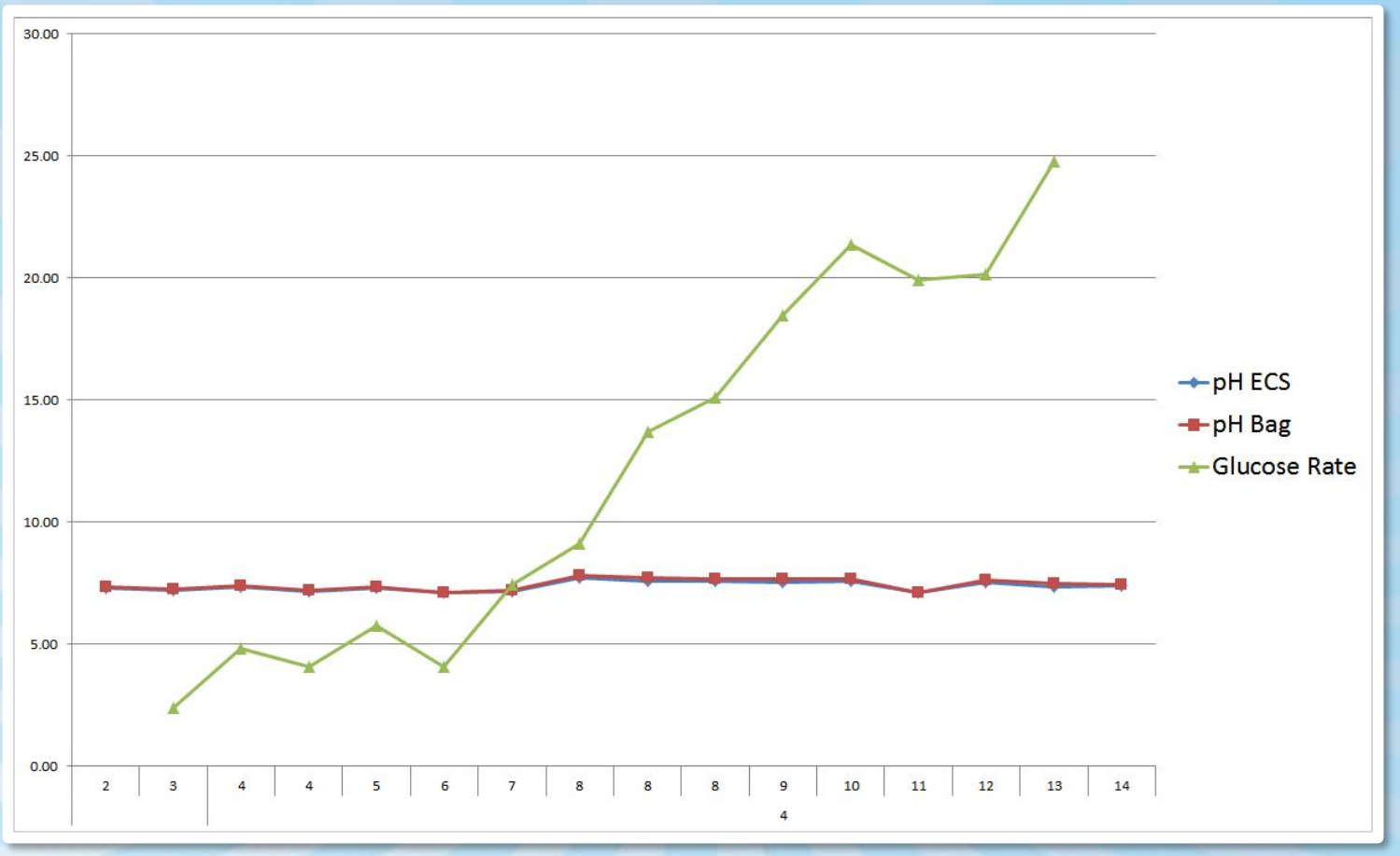

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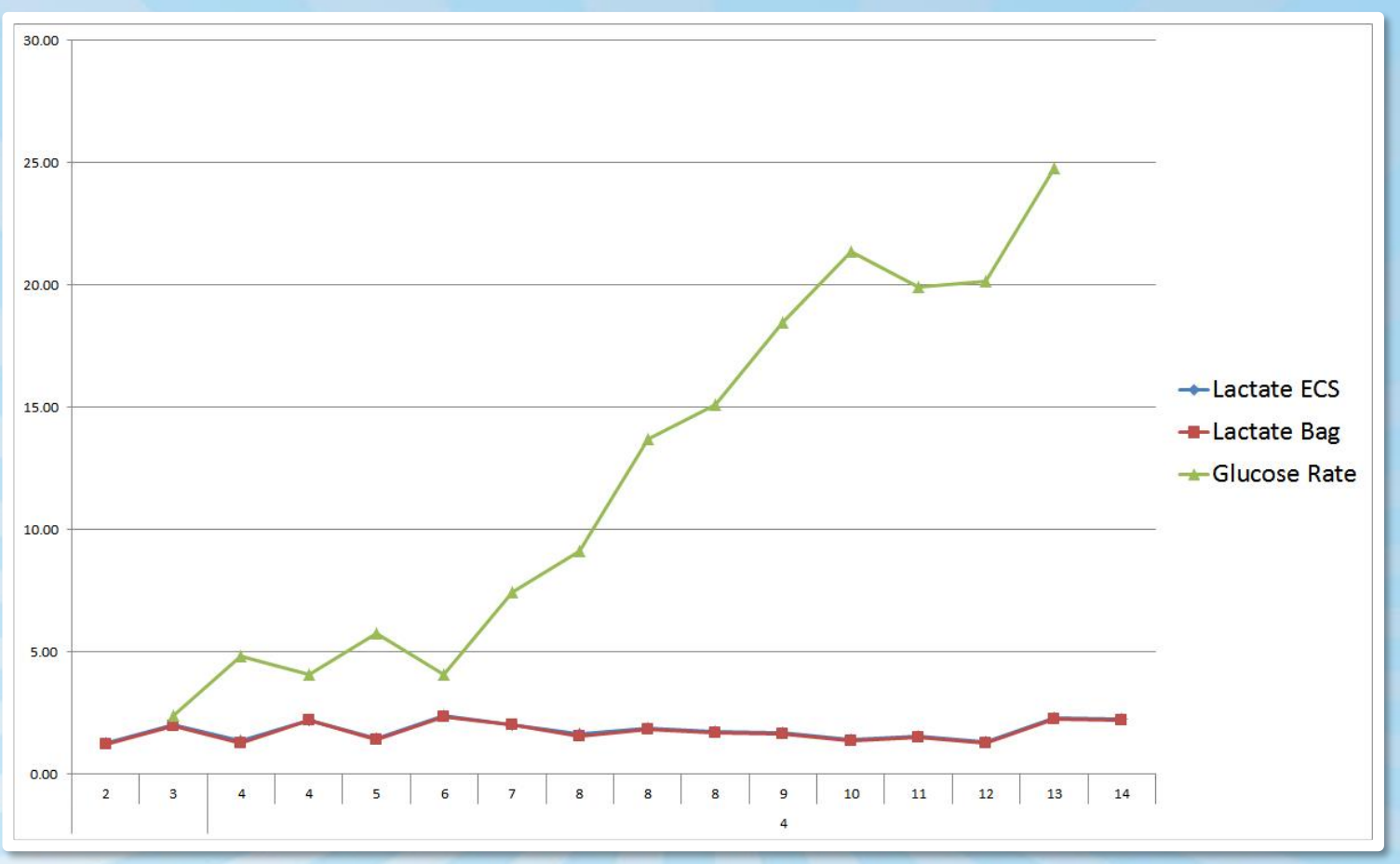

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Uniformity of Cell Distribution in Cartridge

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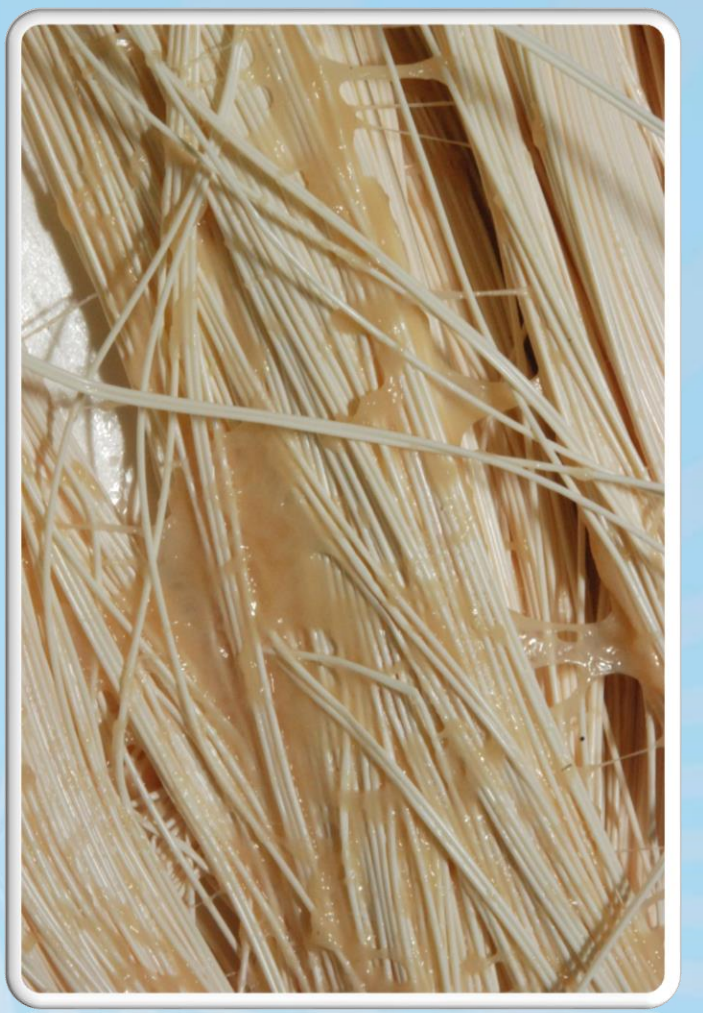

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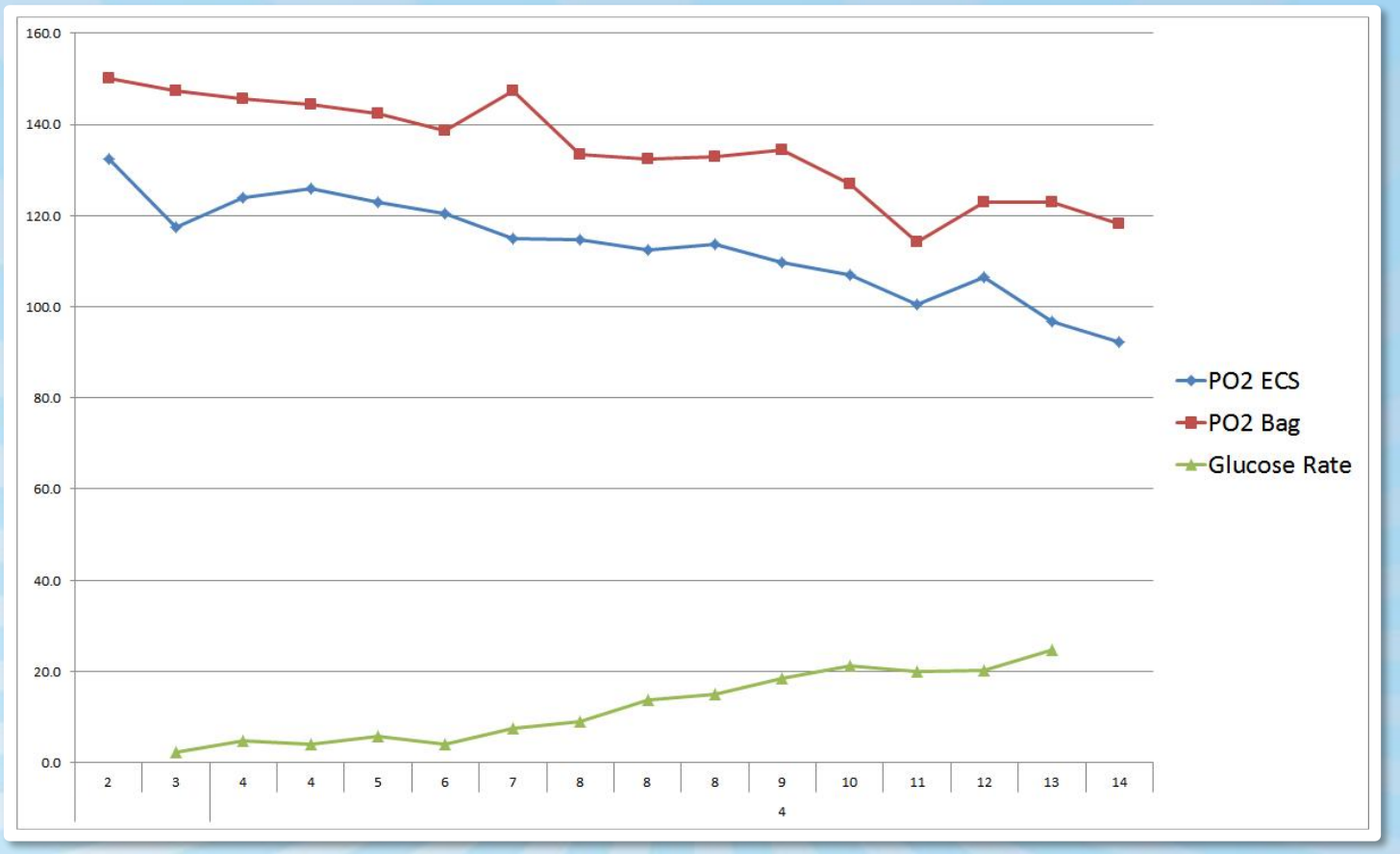

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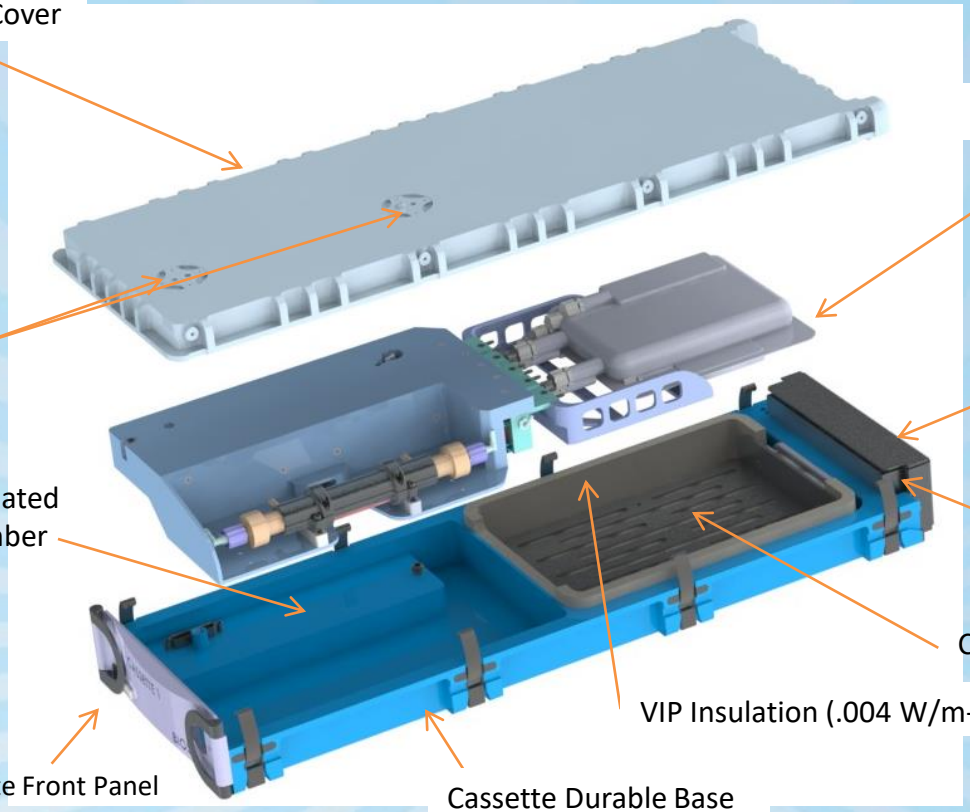
Cassette Assembly



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Trends in Bio-manufacturing

- Single use technology
- Perfusion/continuous production
- Higher productivity cells
- Smaller batch sizes, higher bioactivity
- Proteins becoming more complex

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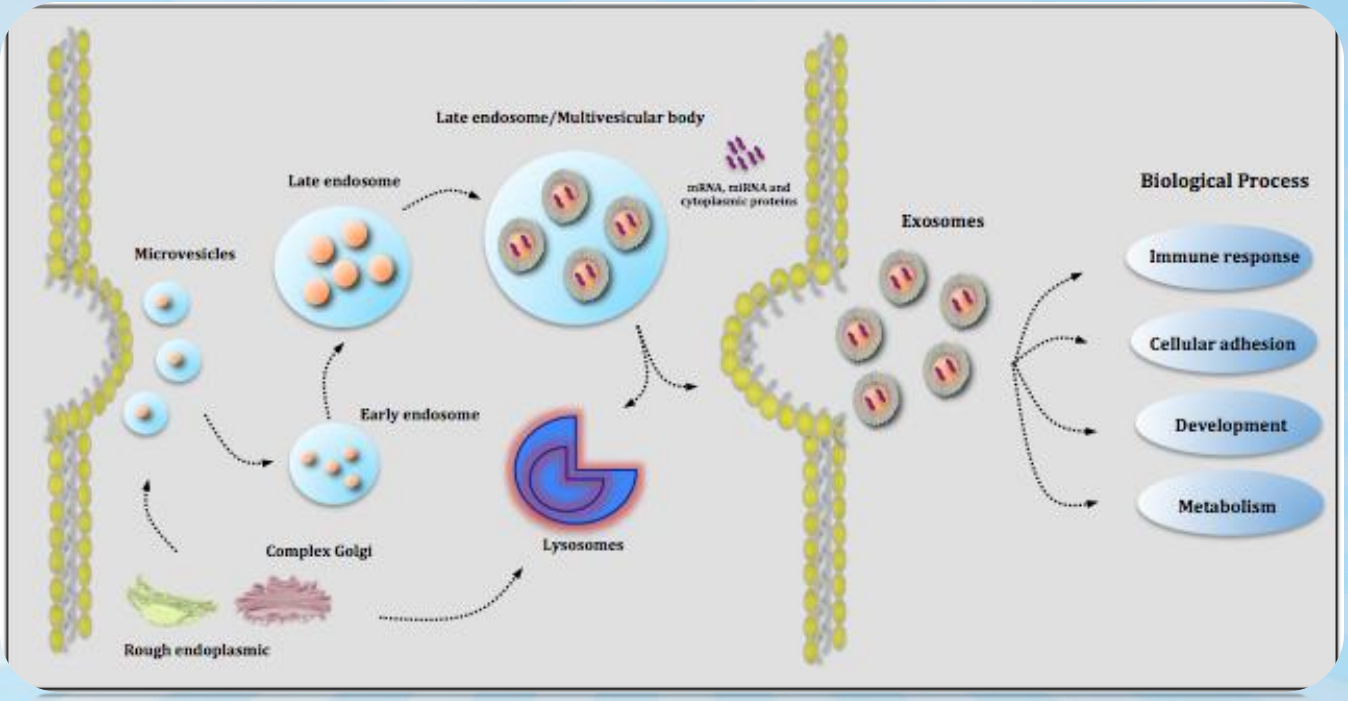
Exosomes

- Cell-derived vesicles in biological fluids
- Including medium of cultured cells
- Dia. between 30 and 100 nm
- Contain cellular proteins and RNA
- Facilitate cell-to-cell transfer of cargo
- May play a role in cell-to-cell signaling
- May mediate adaptive immune responses



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Limitations to Current Methods

- Non-Disposable. Cleaning validation
- Cells in suspension
- Surfactant and effect on KLA
- Dilute product
- Large footprint and expense
- Media, expensive, contains proteins and surfactants
- Bigger the reactor, less uniformity
- Conditions “controlled”

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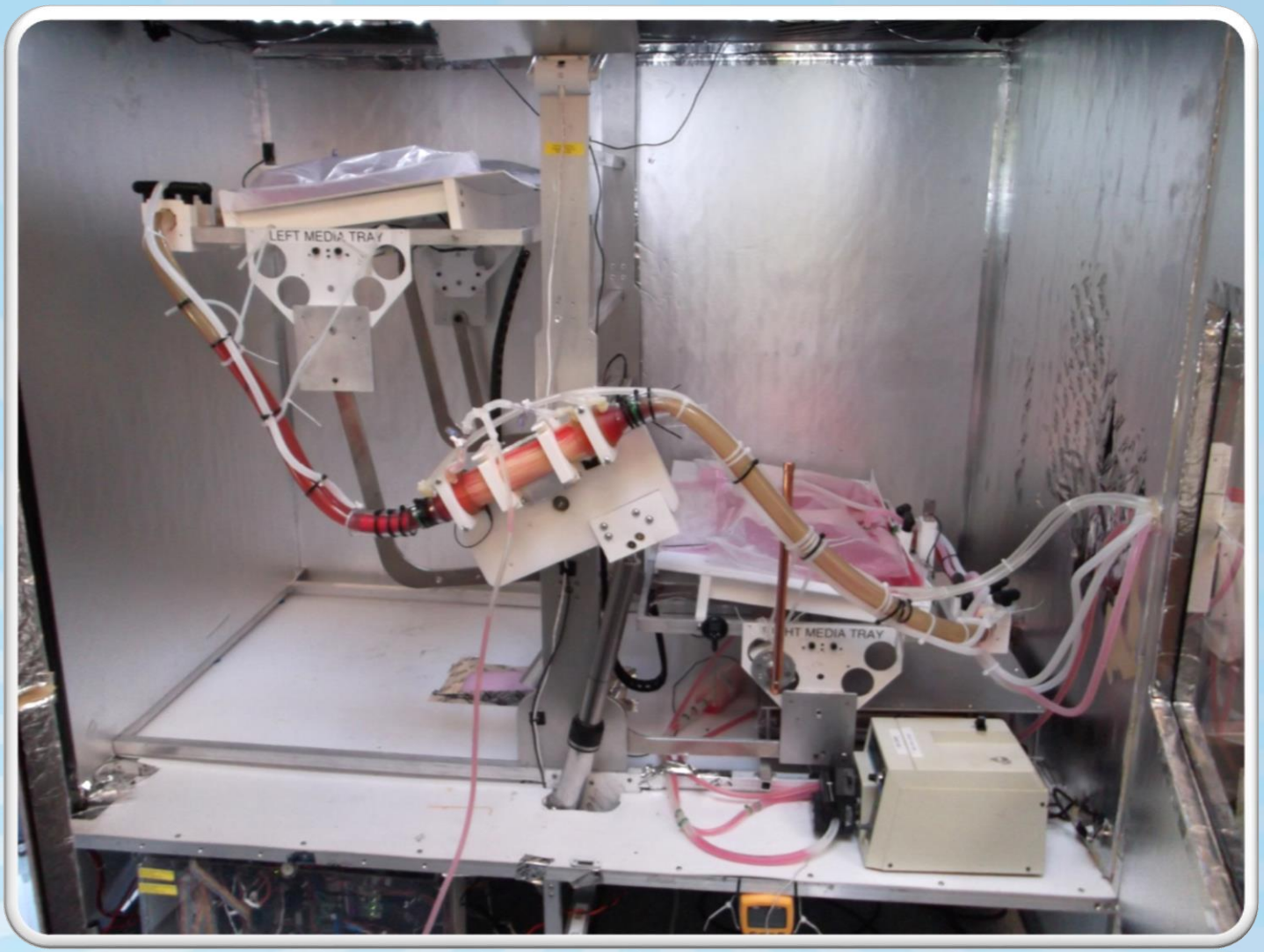

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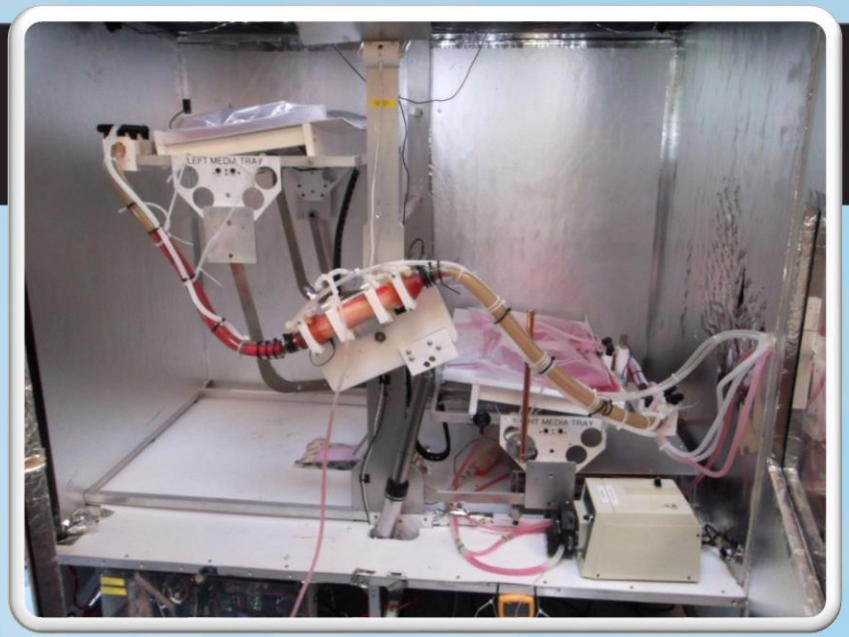
Advantages



- Inexpensive medium
- Small footprint
- Disposable
- No seed reactor
- Enclosed environment
- No adaptation to suspension culture required
- Continuous harvest for labile and toxic proteins
- Small volume of reactor provides increased uniformity throughout the culture volume
- Medium is replaced, not controlled
- Especially suited for complex, highly glycosylated proteins

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- Concentrated product
- Consistent continuous production over time
- Protein free medium
- Single use technology
- Can replace 500 liter to 10,000 liter stirred tanks

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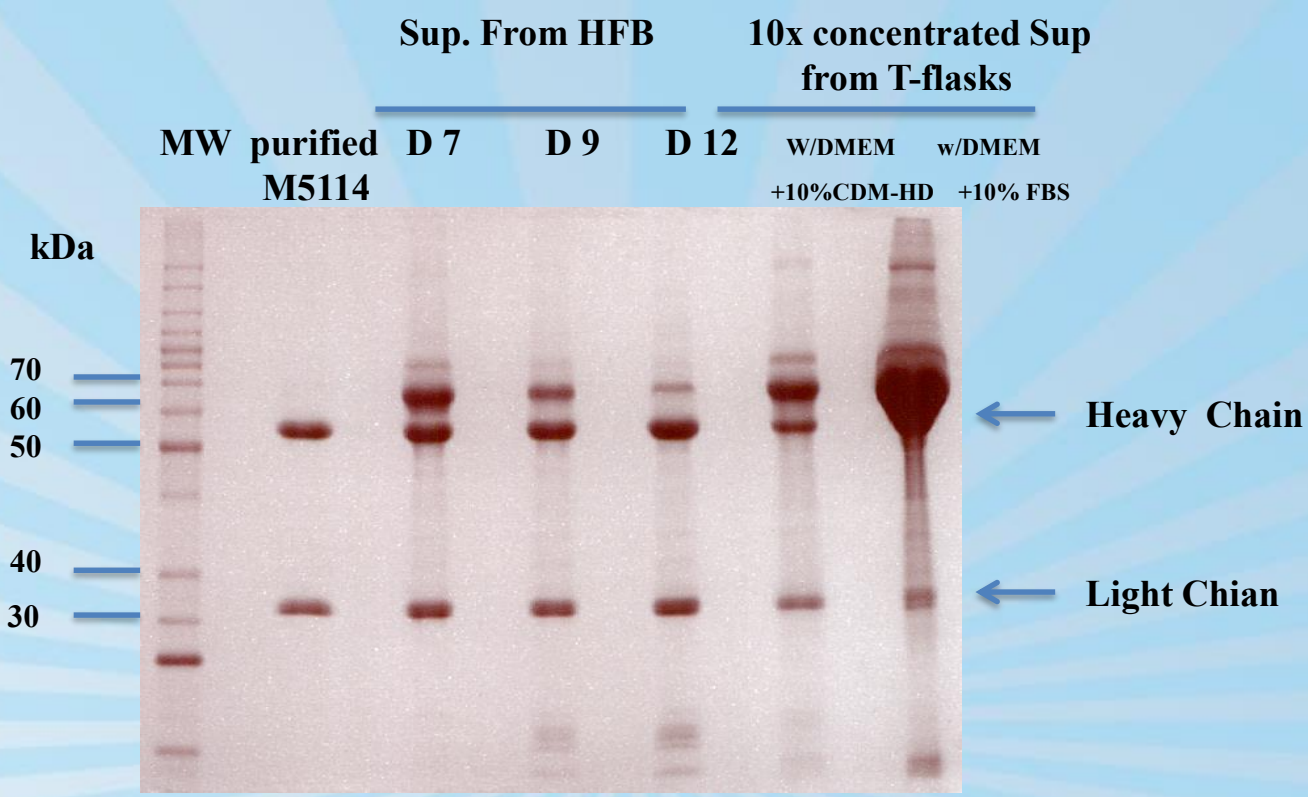

SDS-PAGE analysis of RAb M5114 supernatant

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