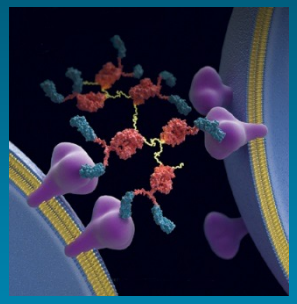
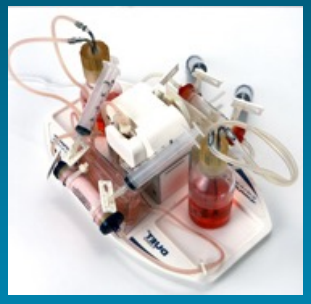
3-D Hollow Fiber Bioreactors: "A Better Way to Grow Cells"





By John J. S. Cadwell

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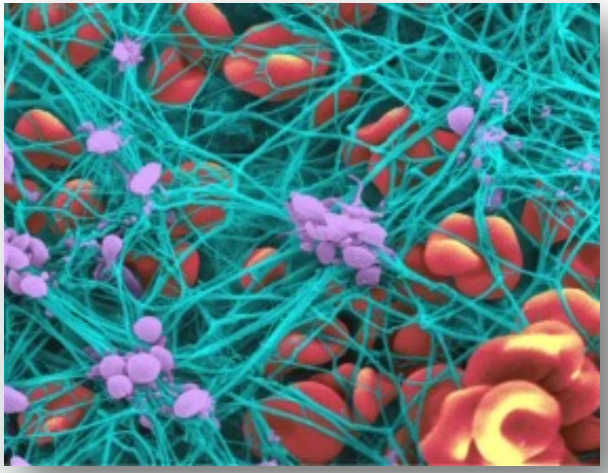
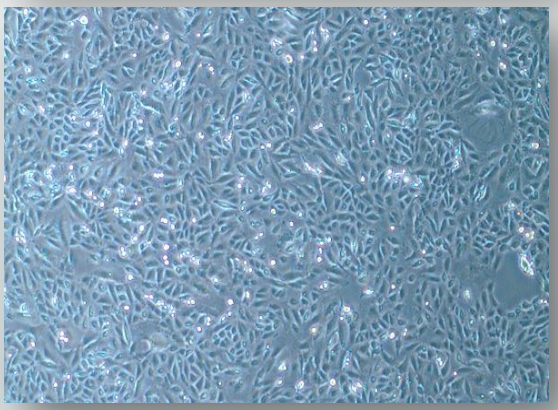
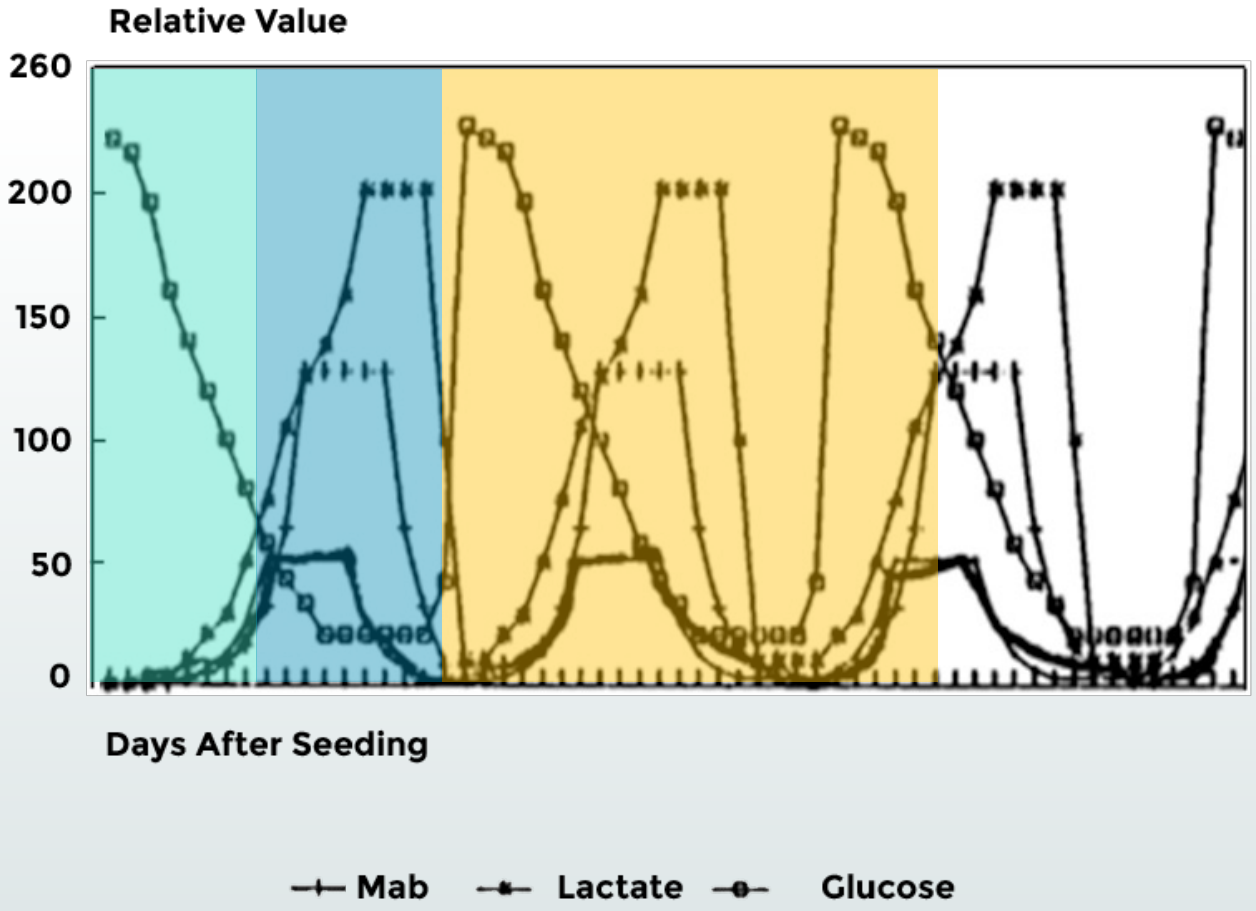
CellME Berlin-International Forum on Cell Manufacturing and Engineering

Cell Culture Through the Ages





“Feast or Famine”



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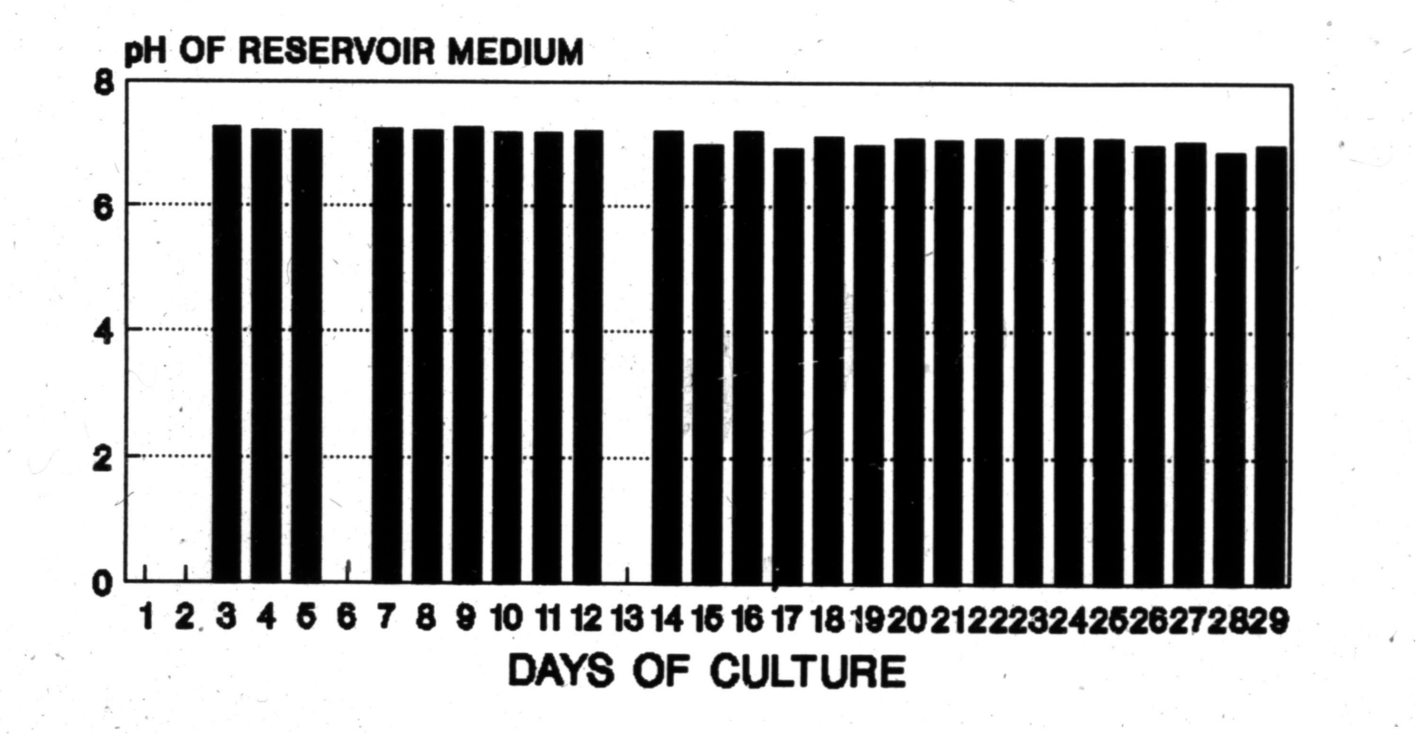
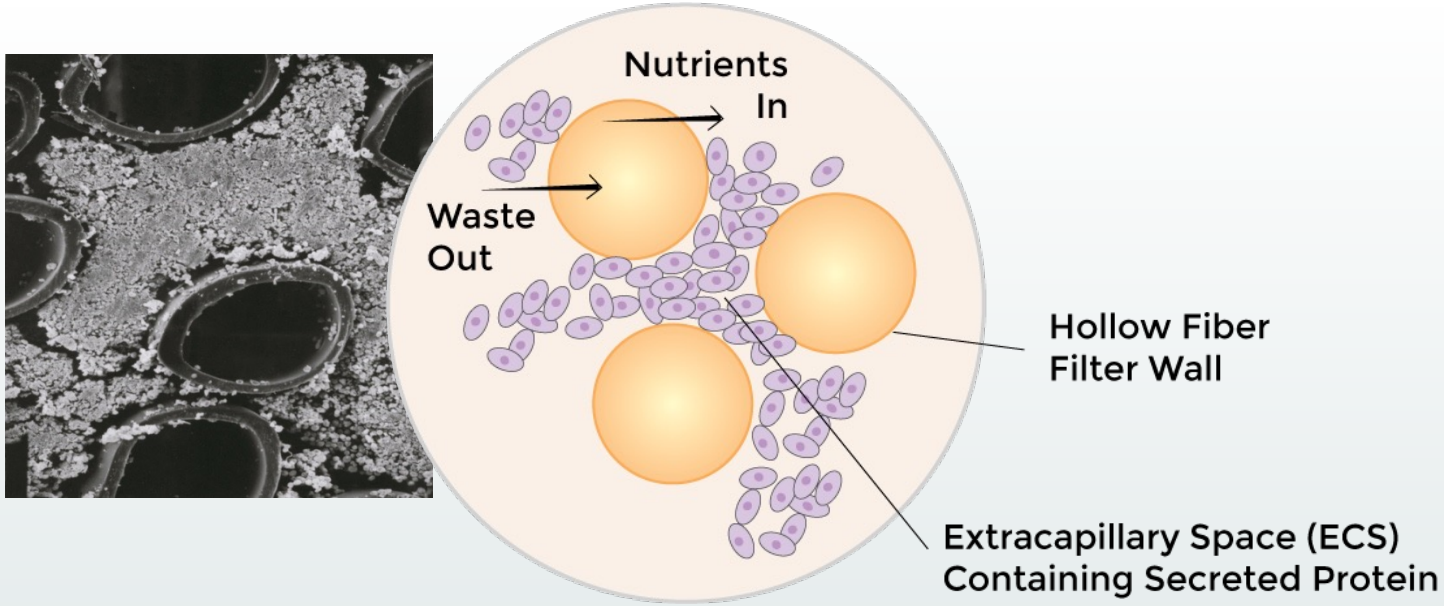
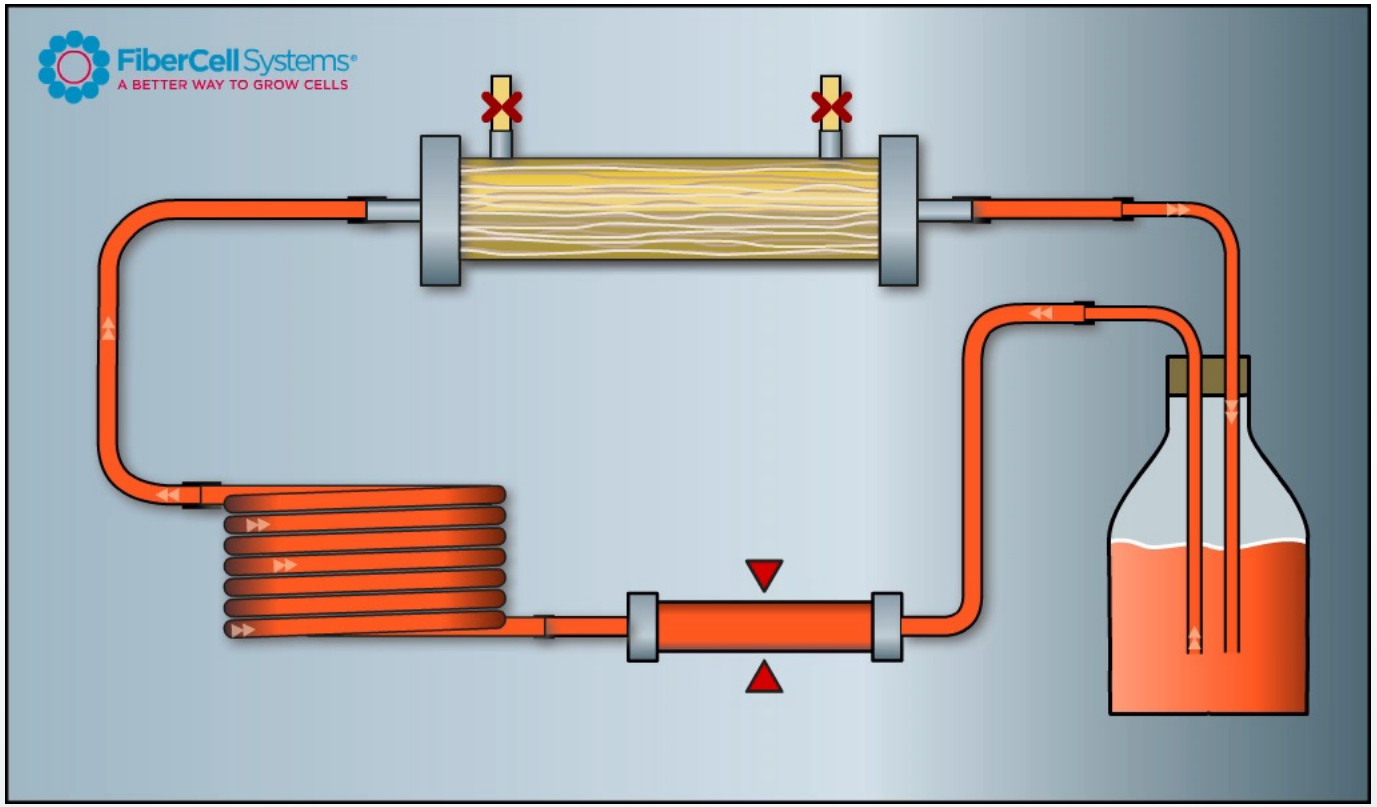
Hollow Fiber Culture of CHO Cells, pH Changes



Hollow Fiber: How it Works





- Positive pressure displacement pump
- Silicone tubing for gas exchange
- Closed, bio-safe system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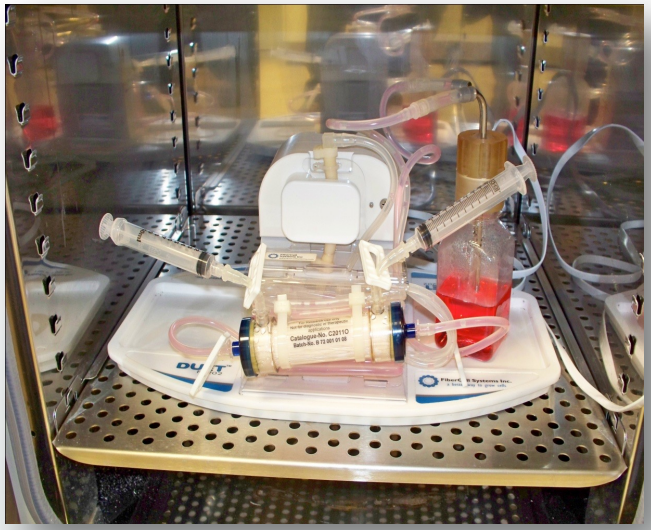
1X PB
Catalog No:
Volume:
Lot No.:
Expires:
Store:
pH:

HFBR are Fundamentally Different in 3 Ways

1. Extremely high surface area/volume permits high density cell culture.
2. Cells are bound to a porous support, not a non-porous 2-D flask.
3. The molecular weight cut off (MWCO) of the fibers retains and concentrates secreted products.

In the Laboratory

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



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

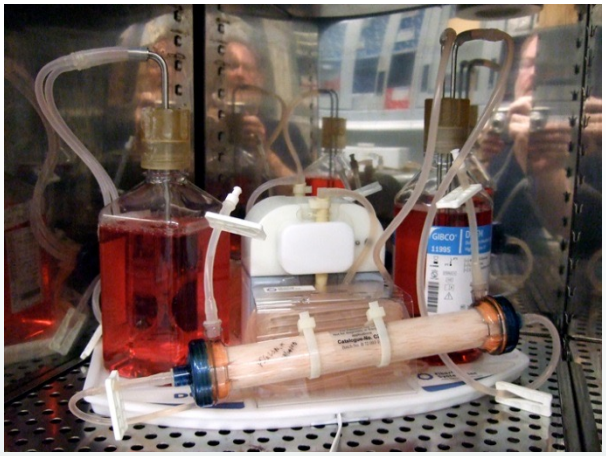
HF Applications

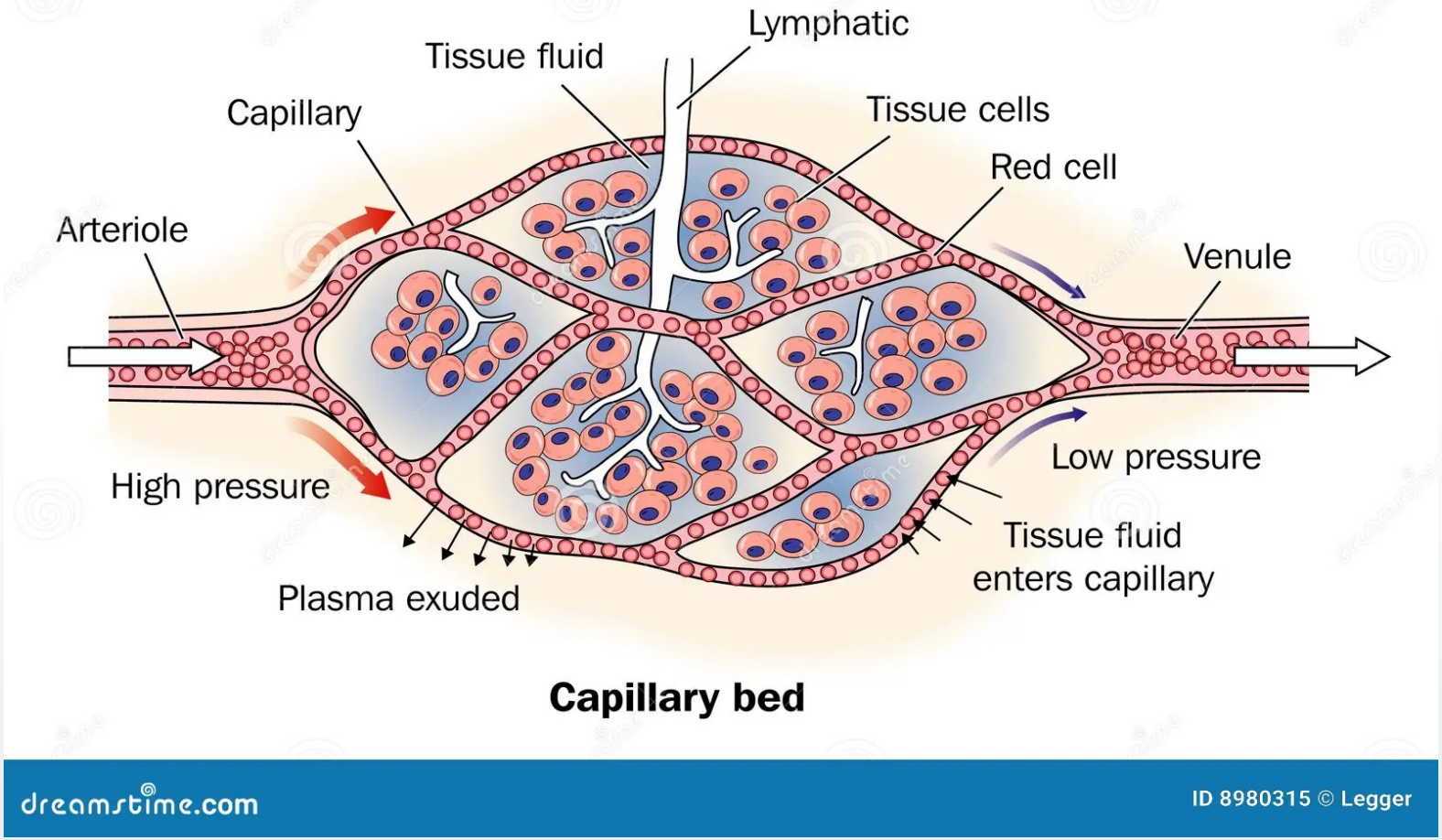
- Monoclonal antibody production
- Recombinant protein production
- Conditioned medium
- Exosome production
- Endothelial cell culture under shear stress
- Cell co-cultivation
- *in vitro* toxicology


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

Advantages of Hollow Fiber Cell Culture

- Concentrated product
- Uniform and complete post-translational modifications
- Low apoptosis, less contamination with intracellular proteins and DNA
- Consistency of production over many months
- Protein-free medium (CDM-HD)





Cytocentric Bioreactor: attempts to recapitulate the *in vivo* microenvironment.

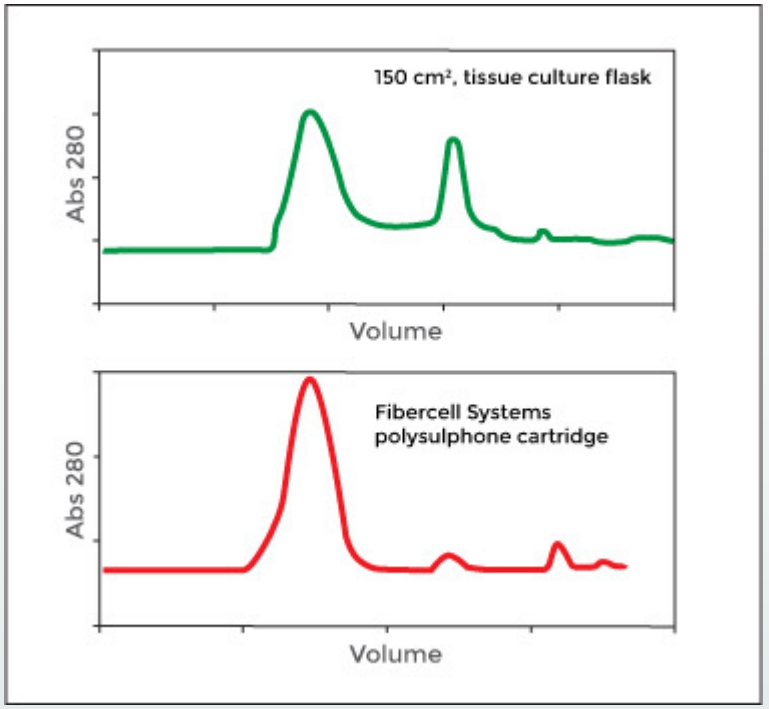
How many different medias are there in the human body?

CDM-HD Serum Replacement

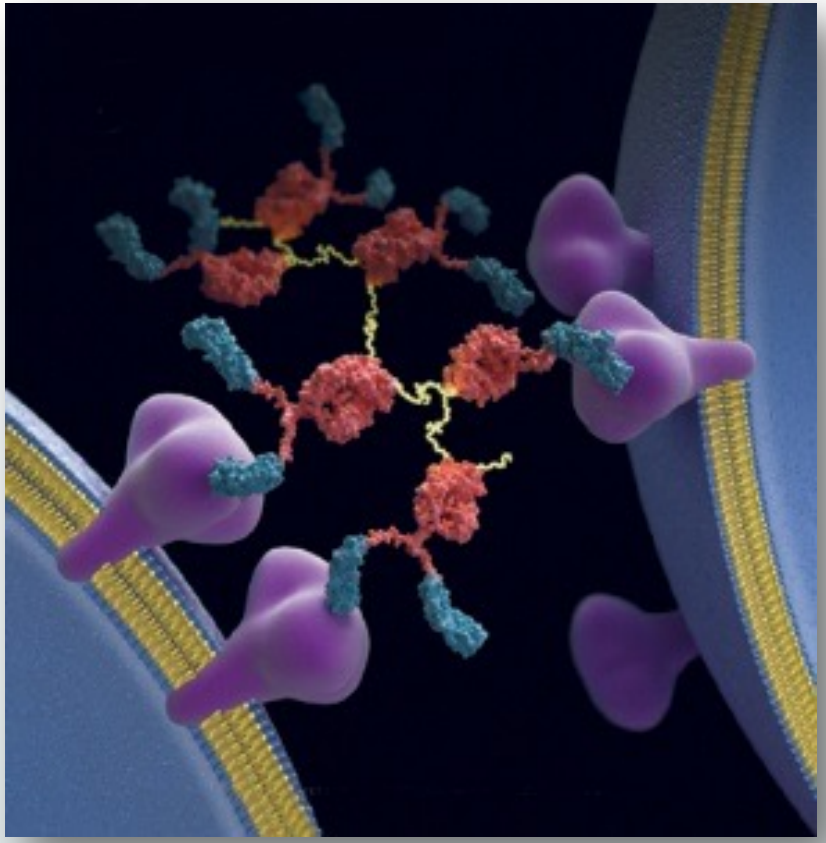
- Optimized and simplified for HFBR
- Contains no surfactants
- Chemically defined, protein-free
- cGMP compliant
- Lot-to-lot consistency
- Ship at ambient, store at 4˚C

Recombinant Protein Production

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



Journal of Biological Chemistry, Sept 2007



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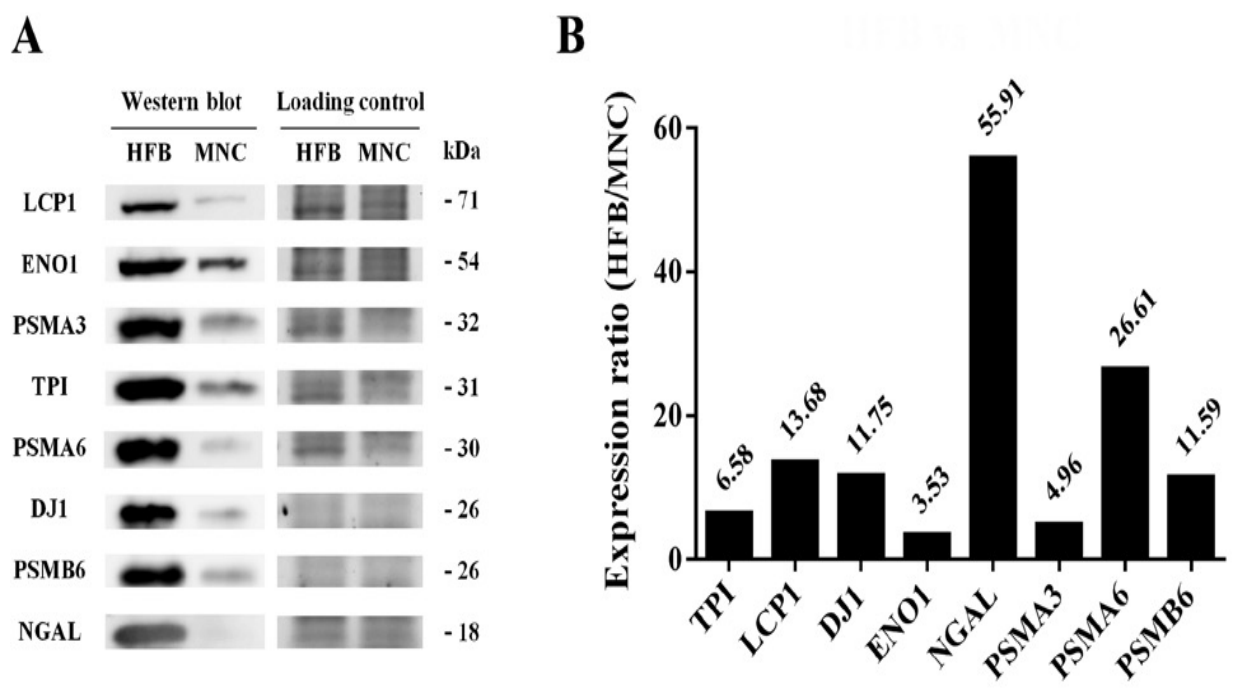
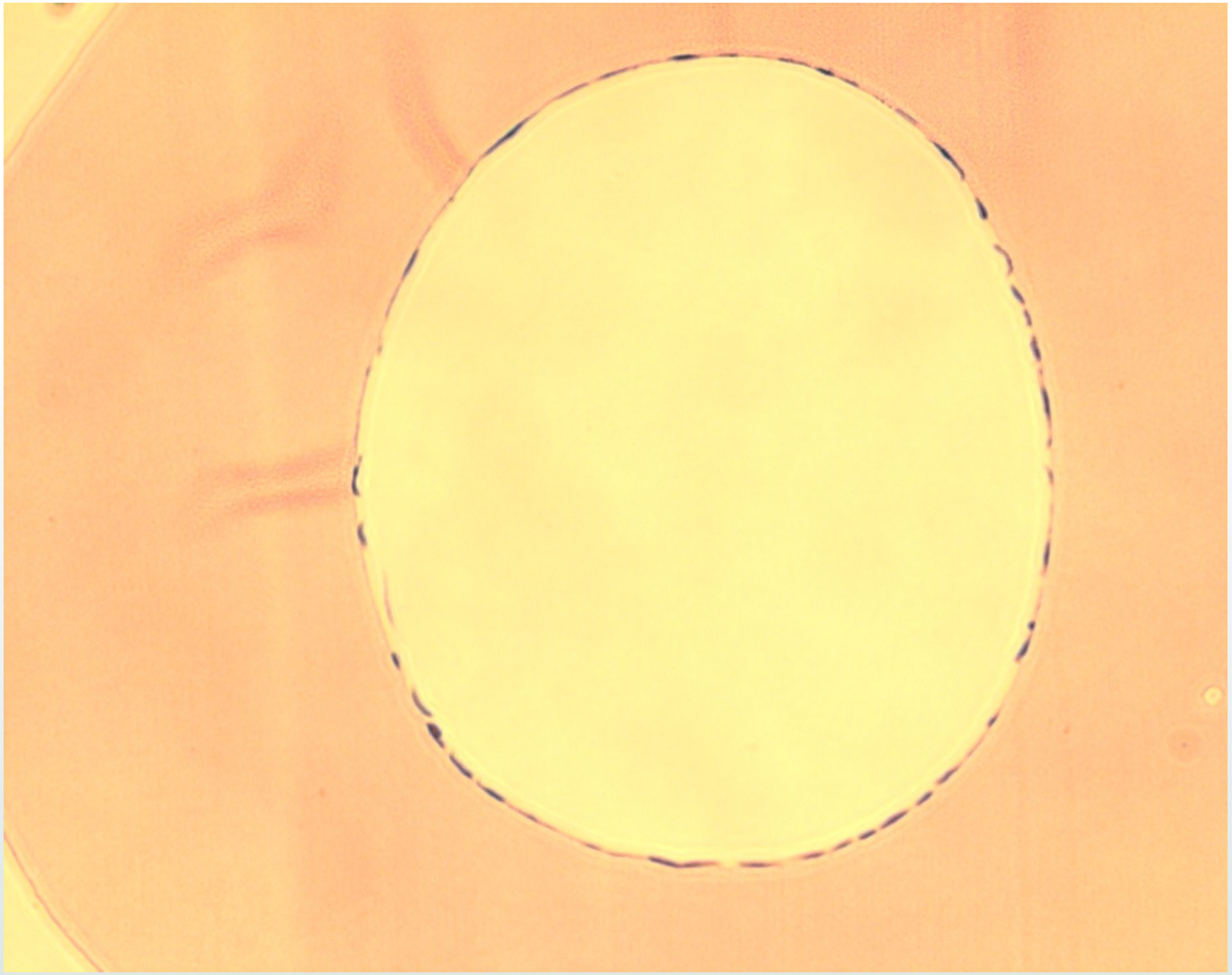
Plastic Waste Generated by $1x10^9$ Cells

Secretome Analysis for Cancer Biomarker Discovery

Comparison of Flask vs. HFBR





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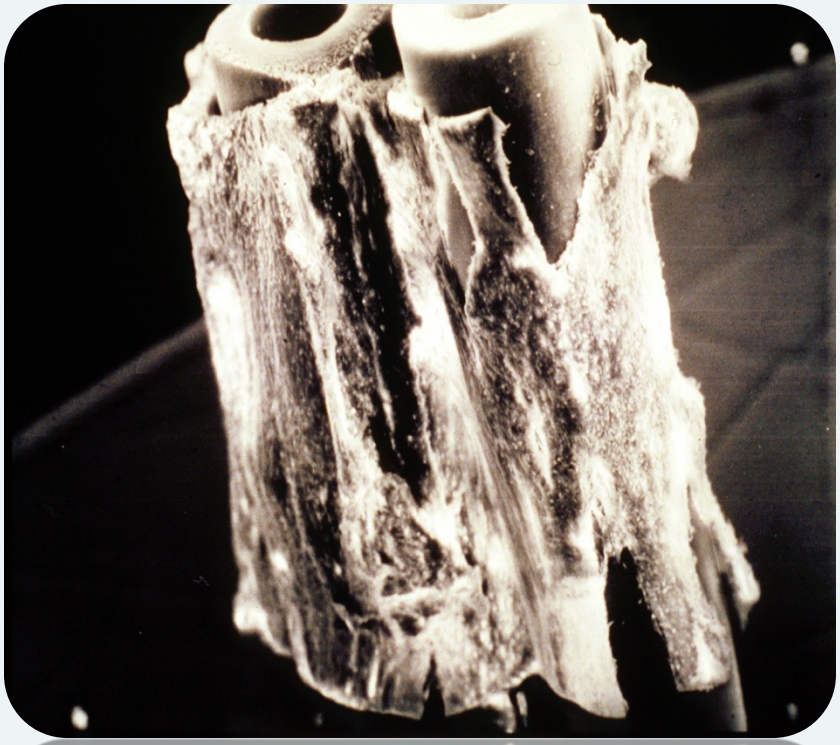
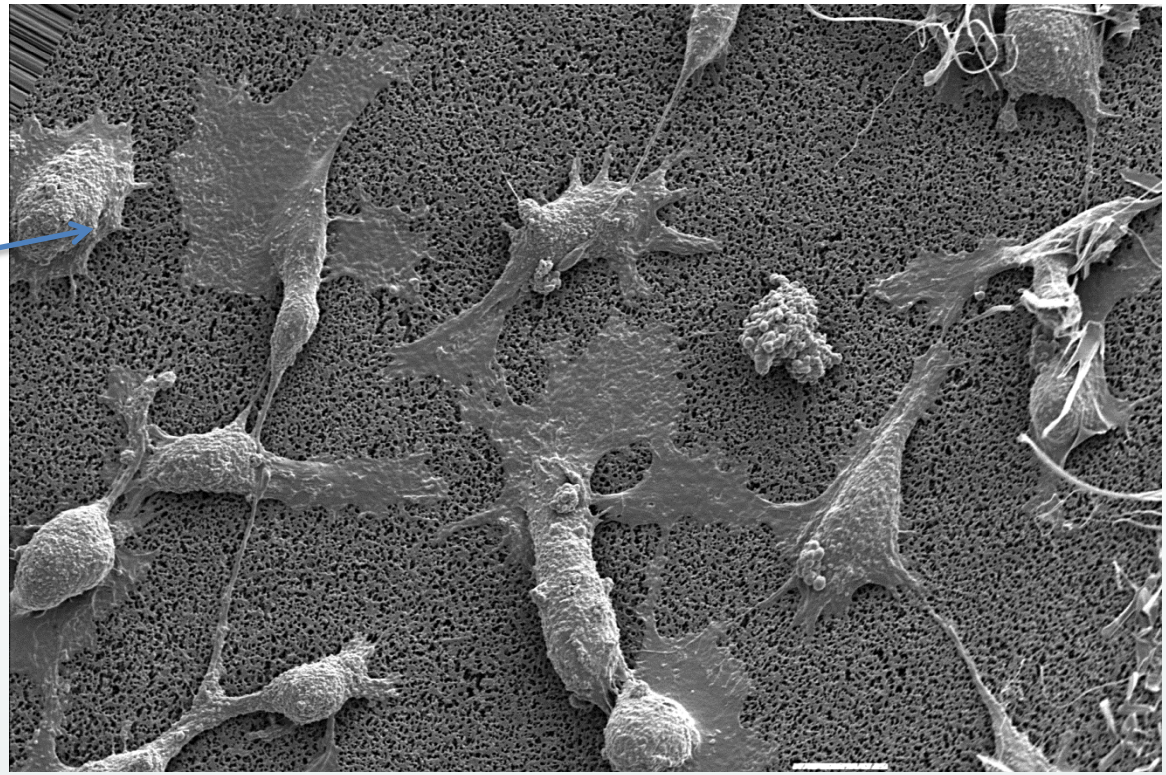
Asymmetric Cell Co-cultivation



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

Pores of fiber

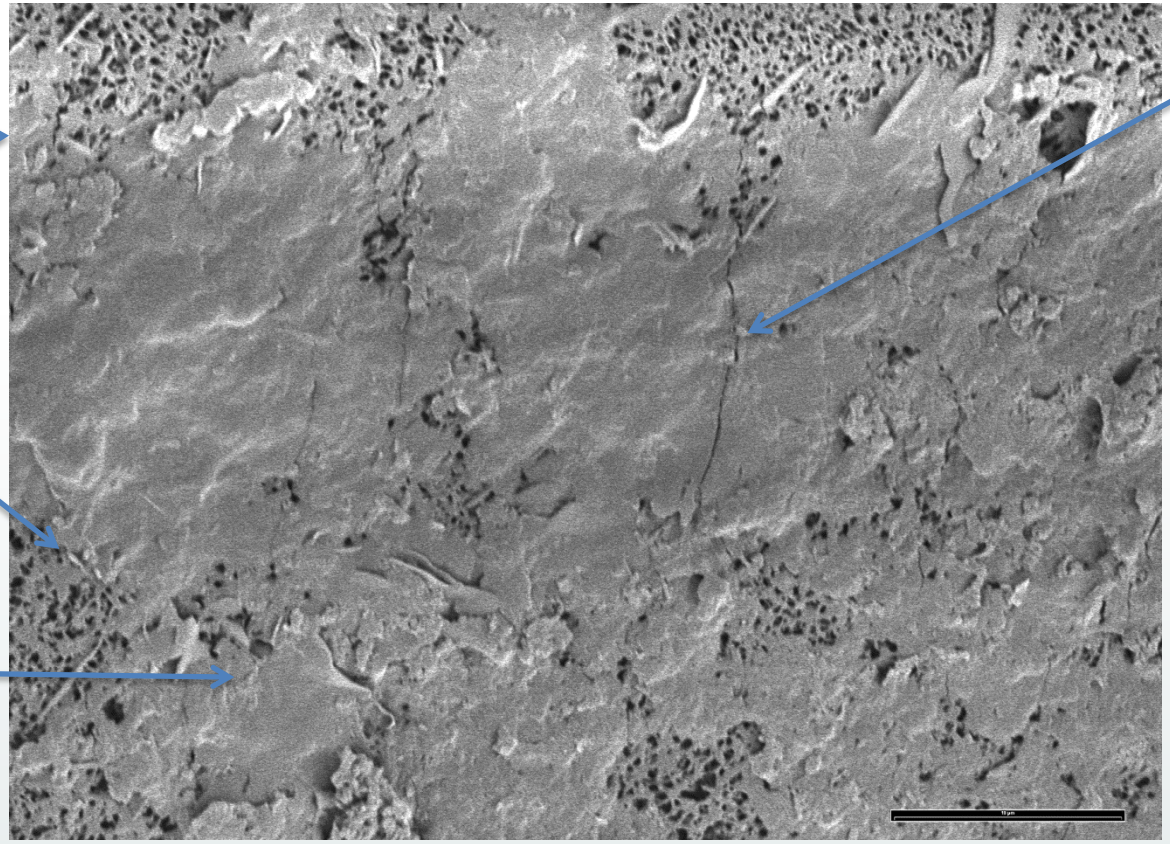


Endothelial cells on the inside on a fiber- these have been adhered to the wall then subjected to very low shear force overnight followed by a few hours at 5 dynes/cm^2.
While the majority of the cells here are still bulky it is possible to see then begin to flatted down onto the wall of the fiber and really stretch out.

Inside of hollow fiber

Pore

Endothelial cell



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

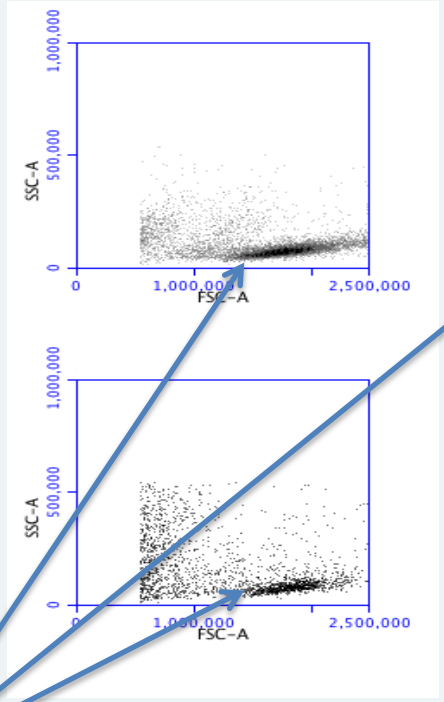
Flattened endothelial cells on inside of fiber. These endothelial cells had been adhered to the inside of the fiber and subjected to minimal shear force over night followed by a minimum of 5 hours at 10 dynes/cm2. This was based on results we saw when testing the application of a different system that 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.

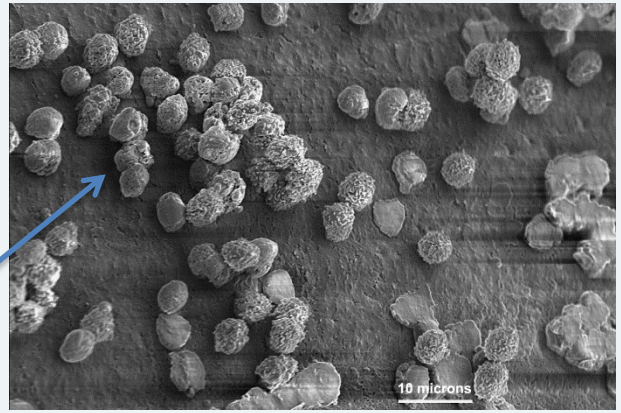
CLL cells actively migrate into the extra-vascular space

Circulating Compartment

Extra-vascular space (migrated compartment)

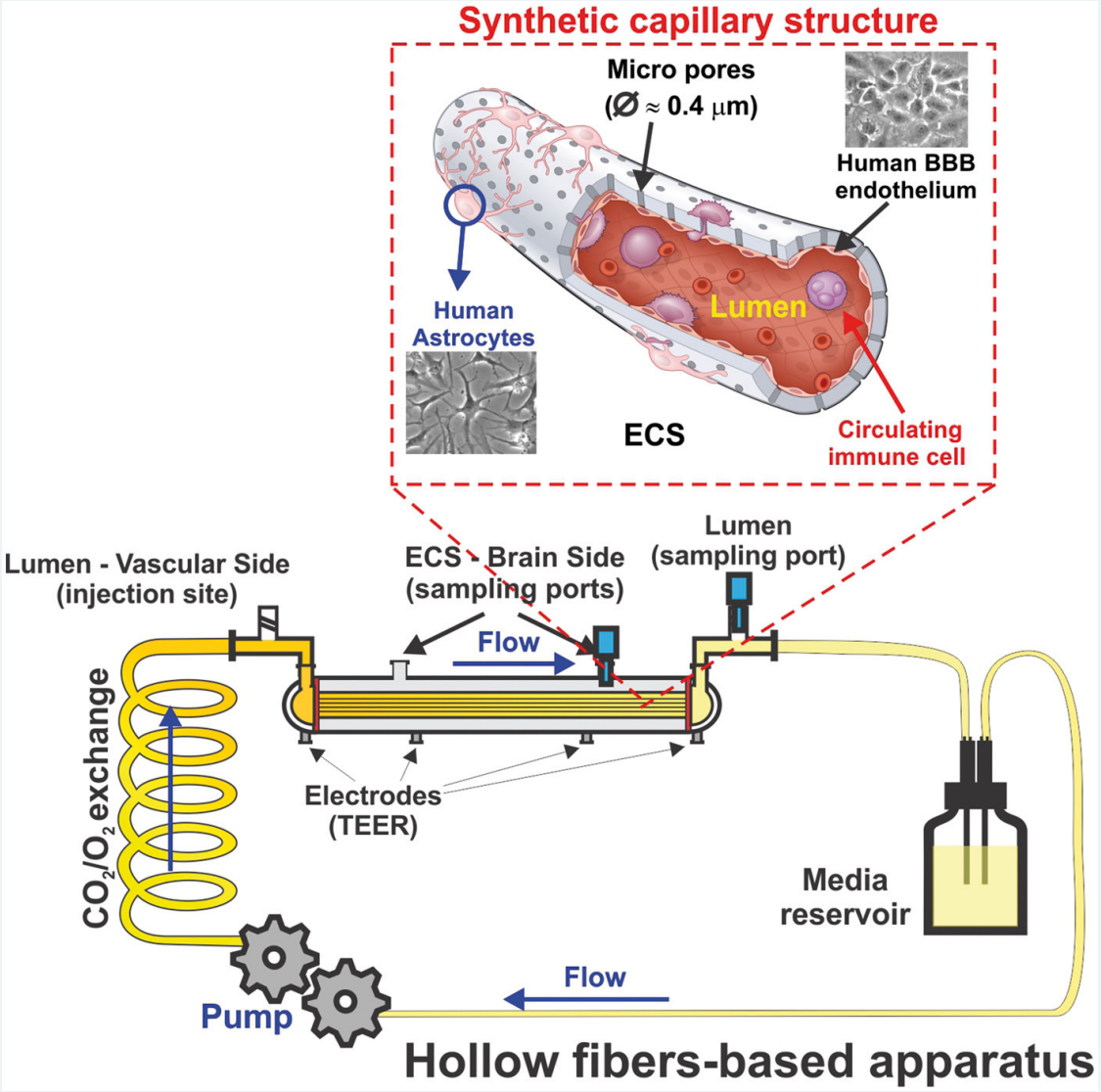
CLL cells



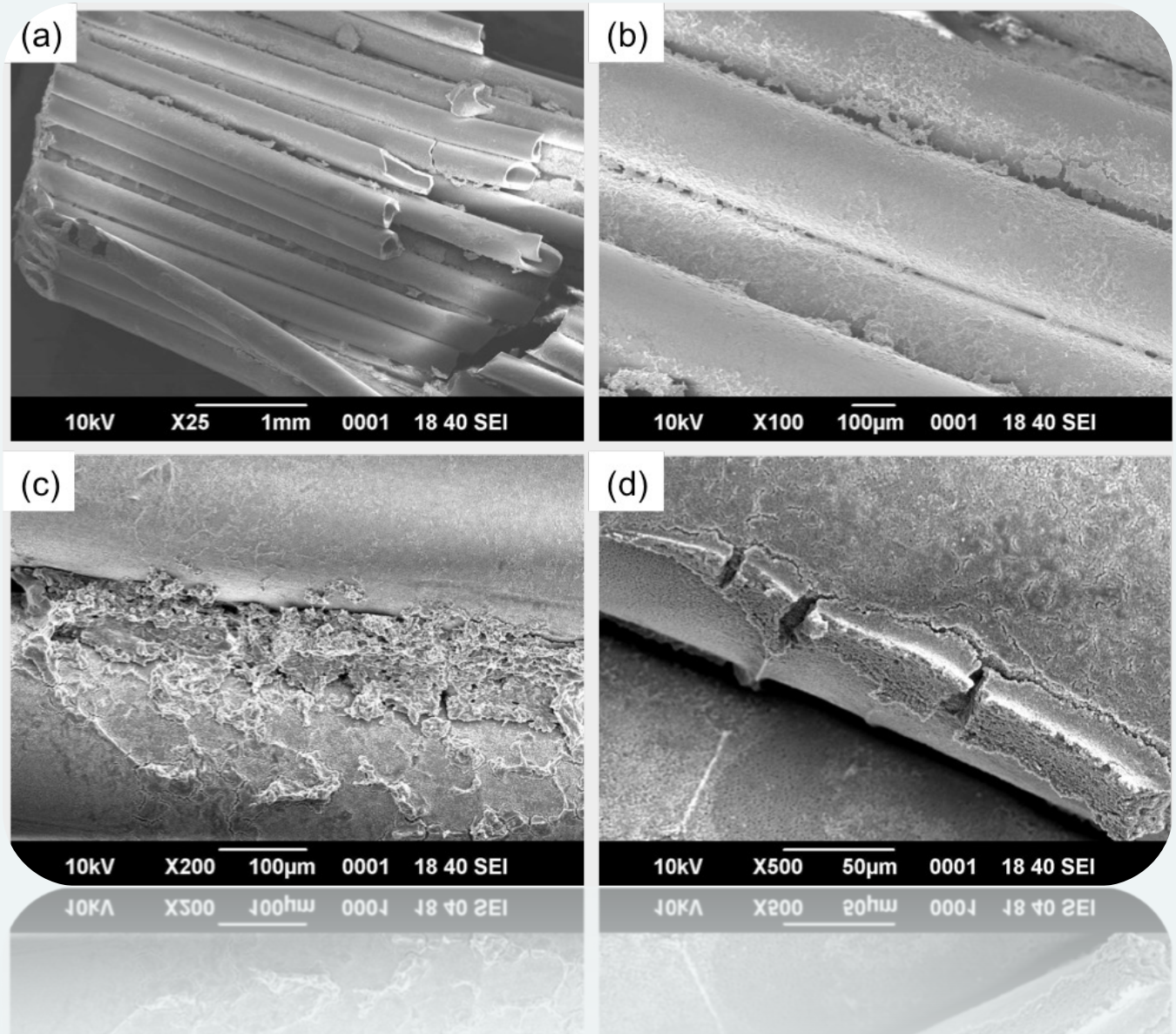


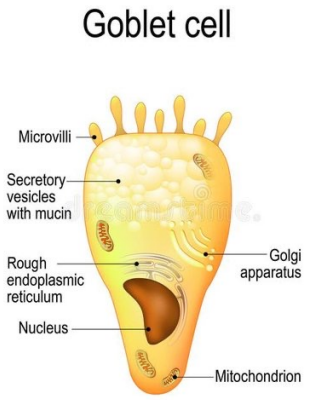
Scanning electron micrograph of the outside of a hollow fibre after circulation of CLL cells around the system

Blood-Brain Barrier Model

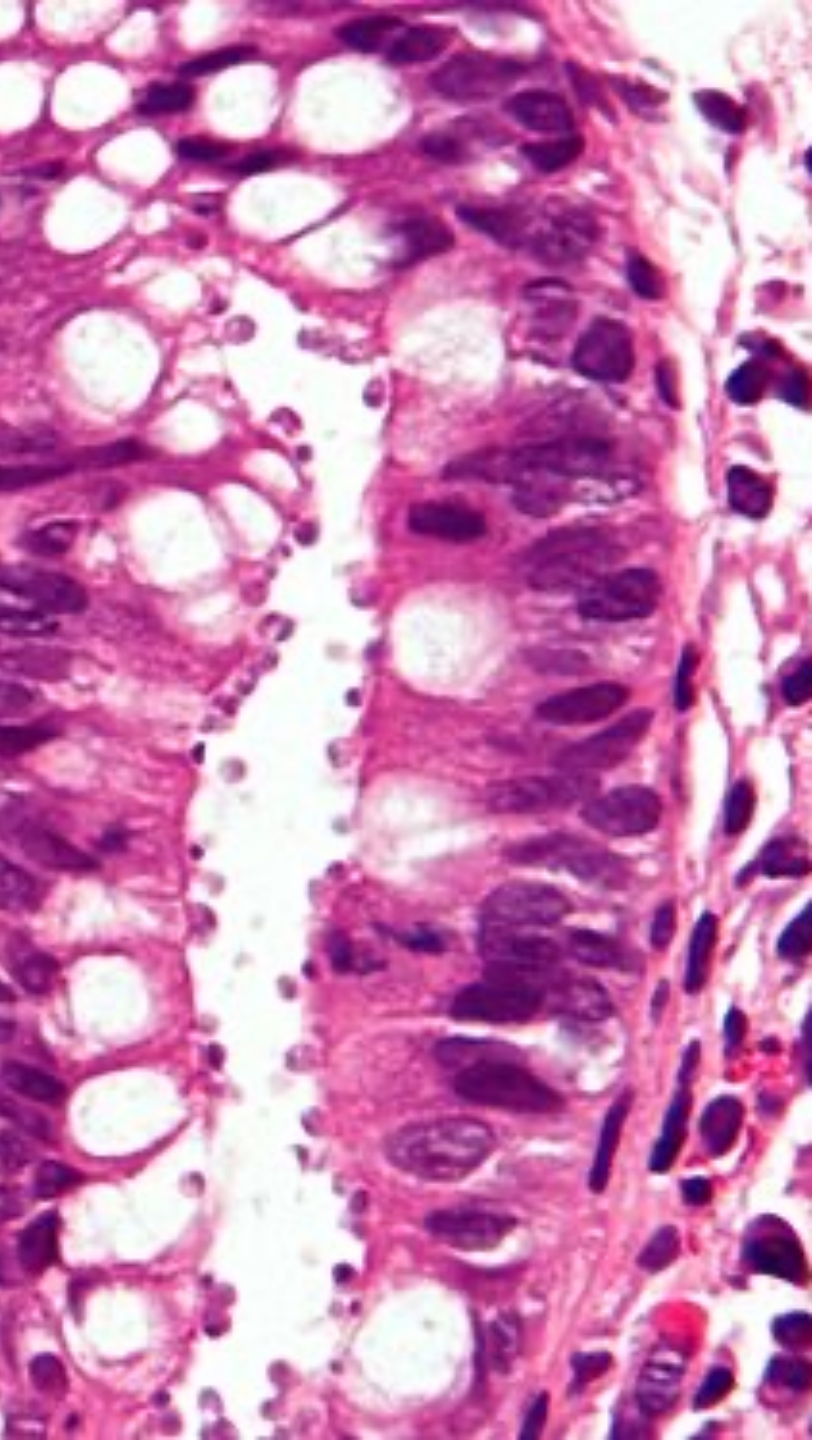


Bone Marrow Stroma/HSC Co-Culture



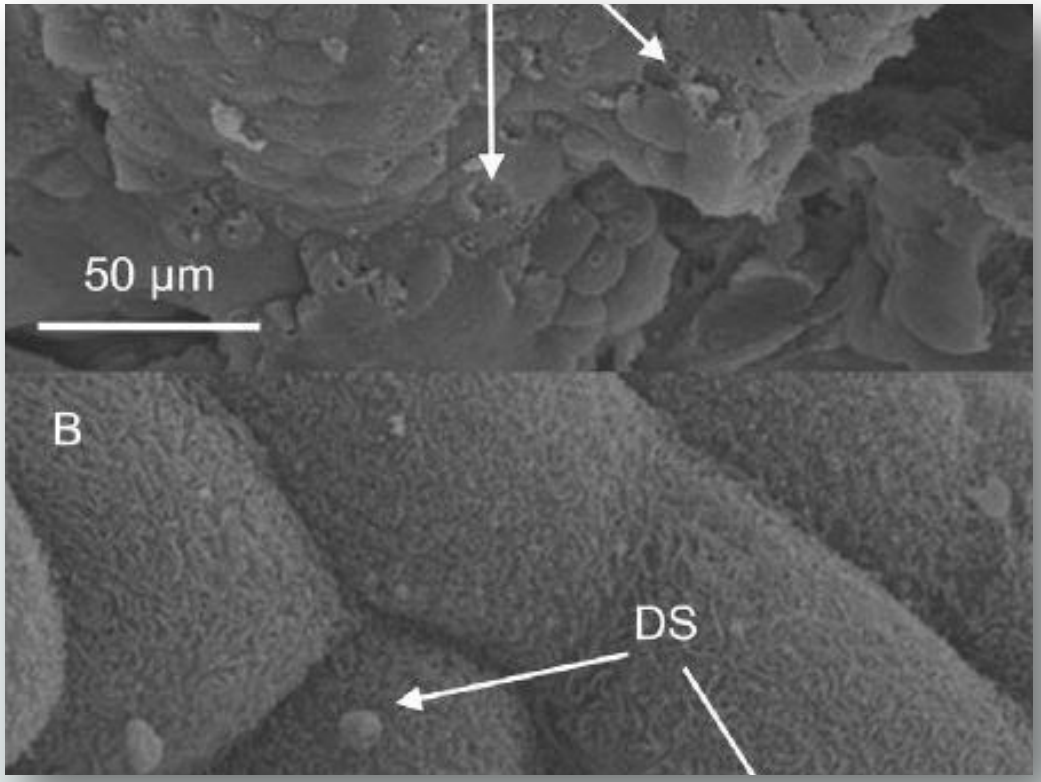


Mixed culture of 85% HCT-8 human intestinal epithelial cells and 15% LS174T goblet cells. Stained with bismark brown to show patches of goblet cells.



Micrograph showing cryptosporidiosis. The cryptosporidium are the small, round bodies in apical vacuoles on the surface of the epithelium. H&E stain. Colonic biopsy.	
Specialty	Infectious disease
Symptoms	Watery diarrhea, nausea, abdominal pain, fever
Causes	Cryptosporidium infection
Risk factors	Immunocompromisation
Prevention	Avoid contaminated water

Cryptosporidium Culture in an Artificial Gut Model

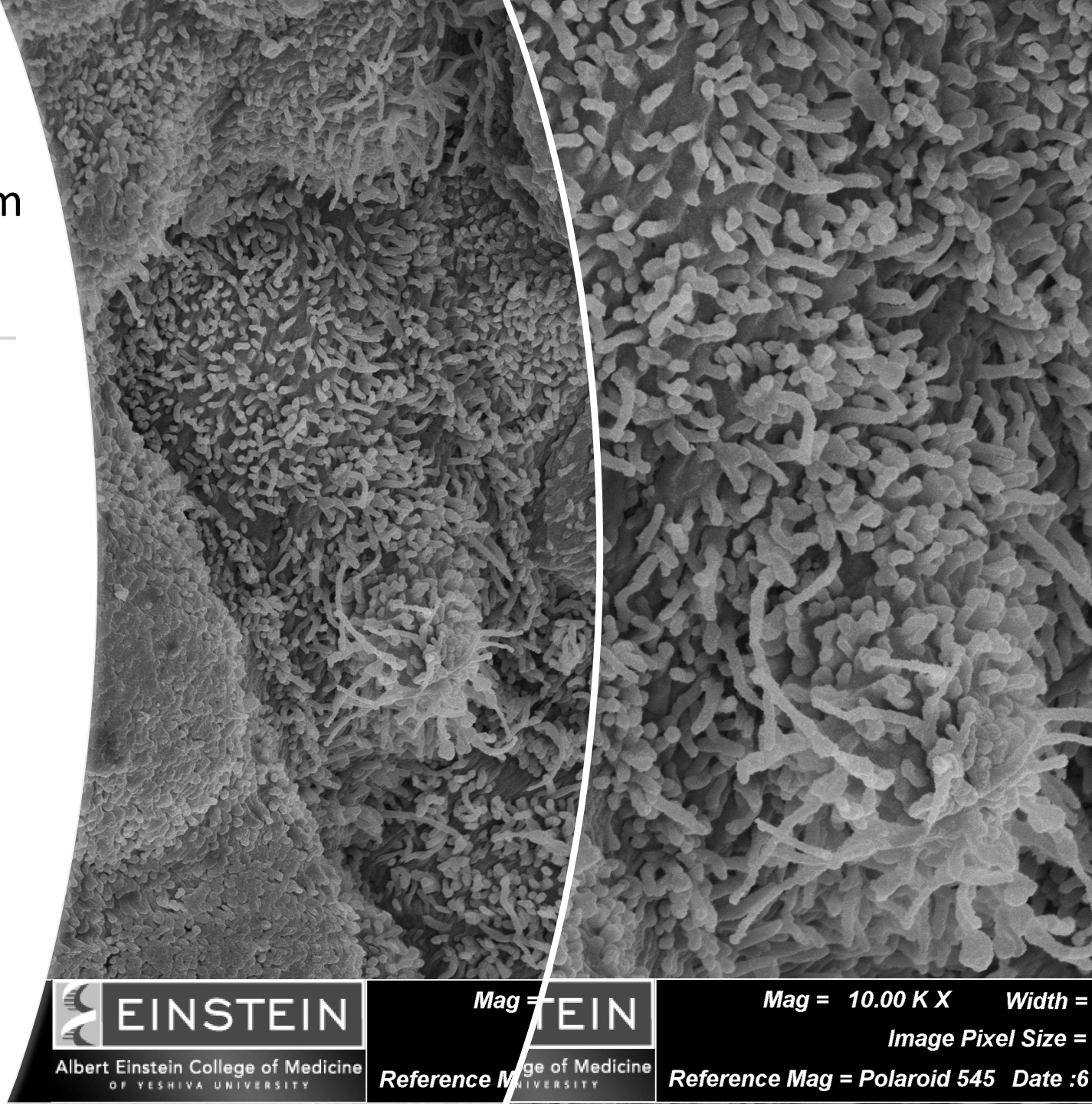


DS: Development stage.

Intracellular but extra-cytoplasmic stage (meront), the enlarged parasitophorous vesicle filled with 8 merozoites which are released when the meront bursts

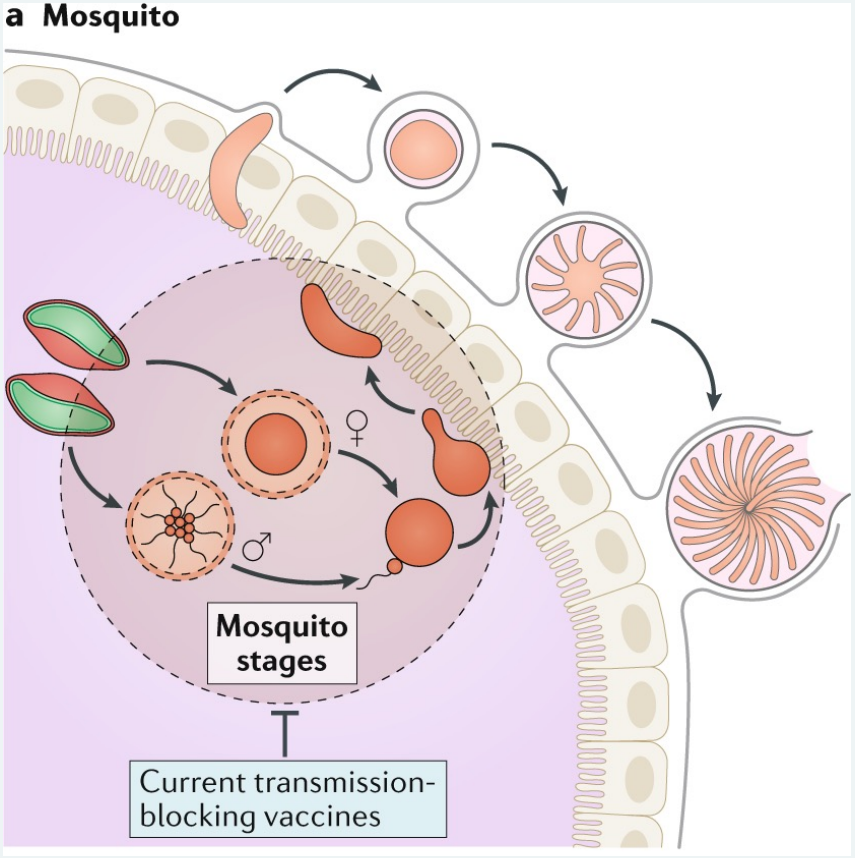
Cryptosporidium

- Intestinal Epithelial cells cultured in 3-D perfusion hollow fiber bioreactor demonstrating polarity and microvilli

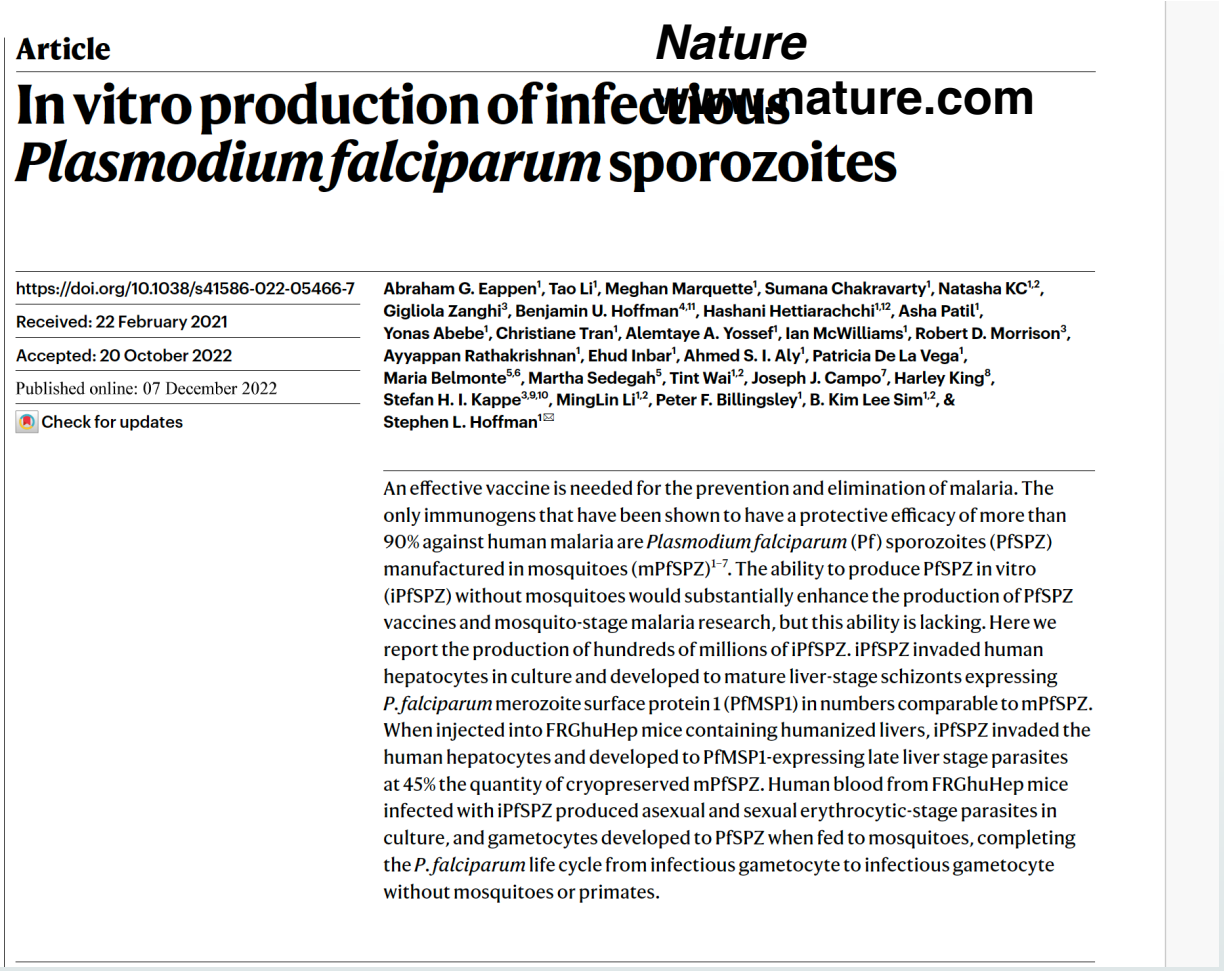


Malaria Vaccine

- Malaria parasites exhibit a complex lifecycle, requiring extensive asexual replication in the liver and blood of the vertebrate host, and in the haemocoel of the insect vector. Yet, they must also undergo a single round of sexual reproduction, which occurs in the vector's midgut upon uptake of a blood meal.



Malaria sporozoites are produced in nature in mosquitoes. In December 2022, a team from Sanaria Inc., a malaria vaccine company, published their groundbreaking working reporting that they could produce infectious *Plasmodium falciparum*



Article

Nature
www.nature.com

In vitro production of infectious *Plasmodium falciparum* sporozoites

https://doi.org/10.1038/s41586-022-05466-7
Received: 22 February 2021
Accepted: 20 October 2022
Published online: 07 December 2022
Check for updates

Abraham G. Eappen[1], Tao Li[1], Meghan Marquette[1], Sumana Chakravarty[1], Natasha KC[1,2], Gigliola Zanghi[3], Benjamin U. Hoffman[4,11], Hashani Hettiarachchi[1,12], Asha Patil[1], Yonas Abebe[1], Christiane Tran[1], Alemtaye A. Yossef[1], Ian McWilliams[1], Robert D. Morrison[3], Ayyappan Rathakrishnan[1], Ehud Inbar[1], Ahmed S. I. Aly[1], Patricia De La Vega[1], Maria Belmonte[5,6], Martha Sedegah[5], Tint Wai[1,2], Joseph J. Campo[7], Harley King[8], Stefan H. I. Kappe[3,9,10], MingLin Li[1,2], Peter F. Billingsley[1], B. Kim Lee Sim[1,2], & Stephen L. Hoffman[1]✉

An effective vaccine is needed for the prevention and elimination of malaria. The only immunogens that have been shown to have a protective efficacy of more than 90% against human malaria are *Plasmodium falciparum* (Pf) sporozoites (PfSPZ) manufactured in mosquitoes (mPfSPZ)[1–7]. The ability to produce PfSPZ in vitro (iPfSPZ) without mosquitoes would substantially enhance the production of PfSPZ vaccines and mosquito-stage malaria research, but this ability is lacking. Here we report the production of hundreds of millions of iPfSPZ. iPfSPZ invaded human hepatocytes in culture and developed to mature liver-stage schizonts expressing *P. falciparum* merozoite surface protein 1 (PfMSP1) in numbers comparable to mPfSPZ. When injected into FRGhuHep mice containing humanized livers, iPfSPZ invaded the human hepatocytes and developed to PfMSP1-expressing late liver stage parasites at 45% the quantity of cryopreserved mPfSPZ. Human blood from FRGhuHep mice infected with iPfSPZ produced asexual and sexual erythrocytic-stage parasites in culture, and gametocytes developed to PfSPZ when fed to mosquitoes, completing the *P. falciparum* life cycle from infectious gametocyte to infectious gametocyte without mosquitoes or primates.

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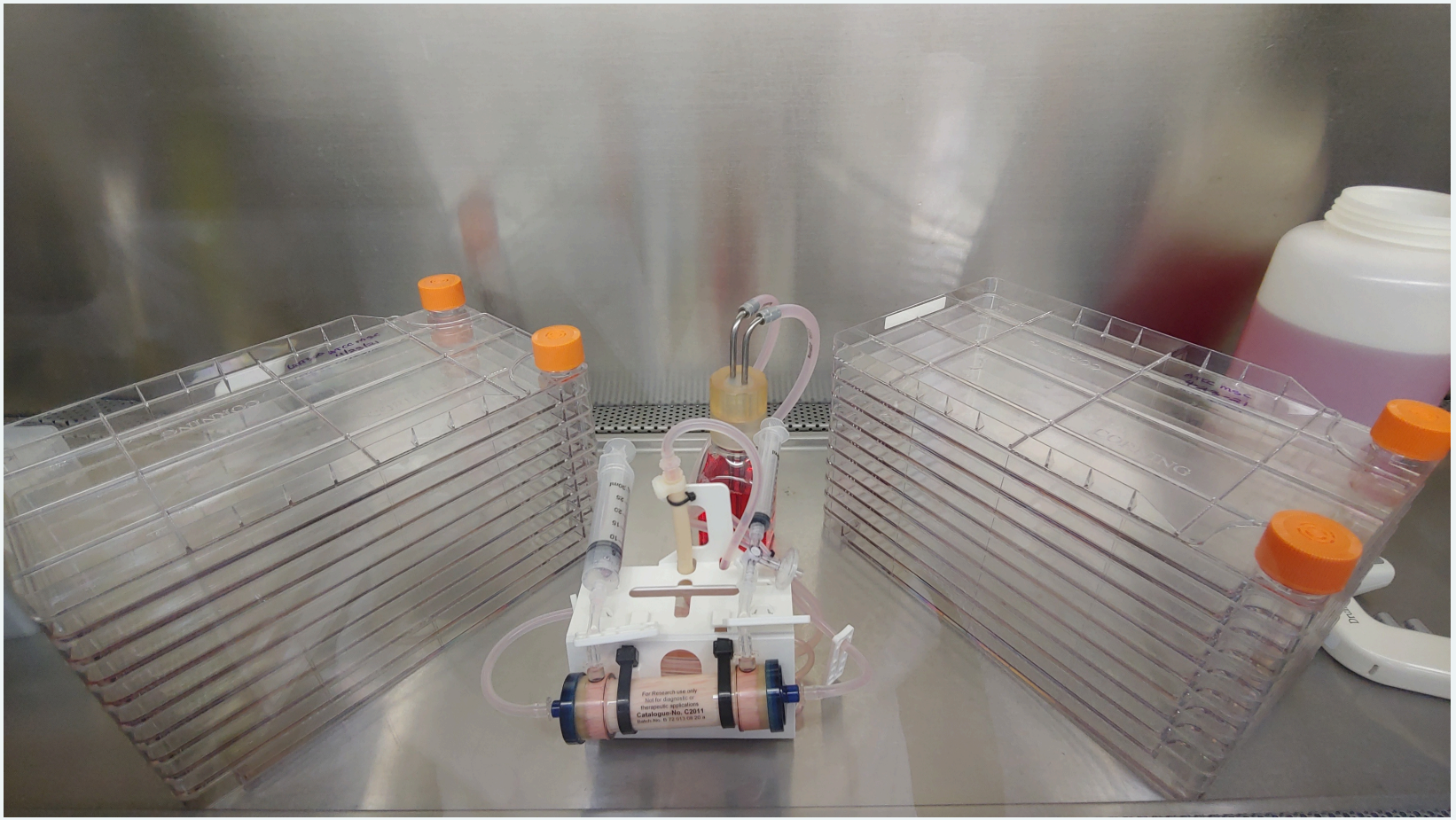
Production of *P. falciparum* sporozoites *in vitro*

- Initial work was done in 8-well chamber slides.
- Next they were cultured in 12 well plates.
- After demonstrating that fully infectious *P. falciparum* sporozoites could be produced *in vitro* without mosquitoes, the Sanaria team recognized that to move to a commercial scale they would have to scale up the process of manufacturing iPfSPZ.
- They were then successful in producing iPfSPZ in Fiber Cell Systems hollow fiber cartridges (HFCs).
- iPfSPZ produced in HFCs were assessed for gene expression by RNA seq, protein expression by immunoblot, and T cell immunogenicity in mice, and the results reported in the *Nature* paper.

Meat and Milk Production

MSCs are plastic adherent fibroblastic cells with the “trilineage potential” of osteogenic, chondrogenic and adipogenic differentiation capabilities. Furthermore, they express the cell surface markers CD73, CD90, and CD105, and do not express haematopoietic and endothelial antigens (CD14 or CD11b, CD19 or CD79α, CD34, CD45, HLA-DR)

Dominici M, Le Blanc K, Mueller I, et al. et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy*. 2006;8(4):315–317



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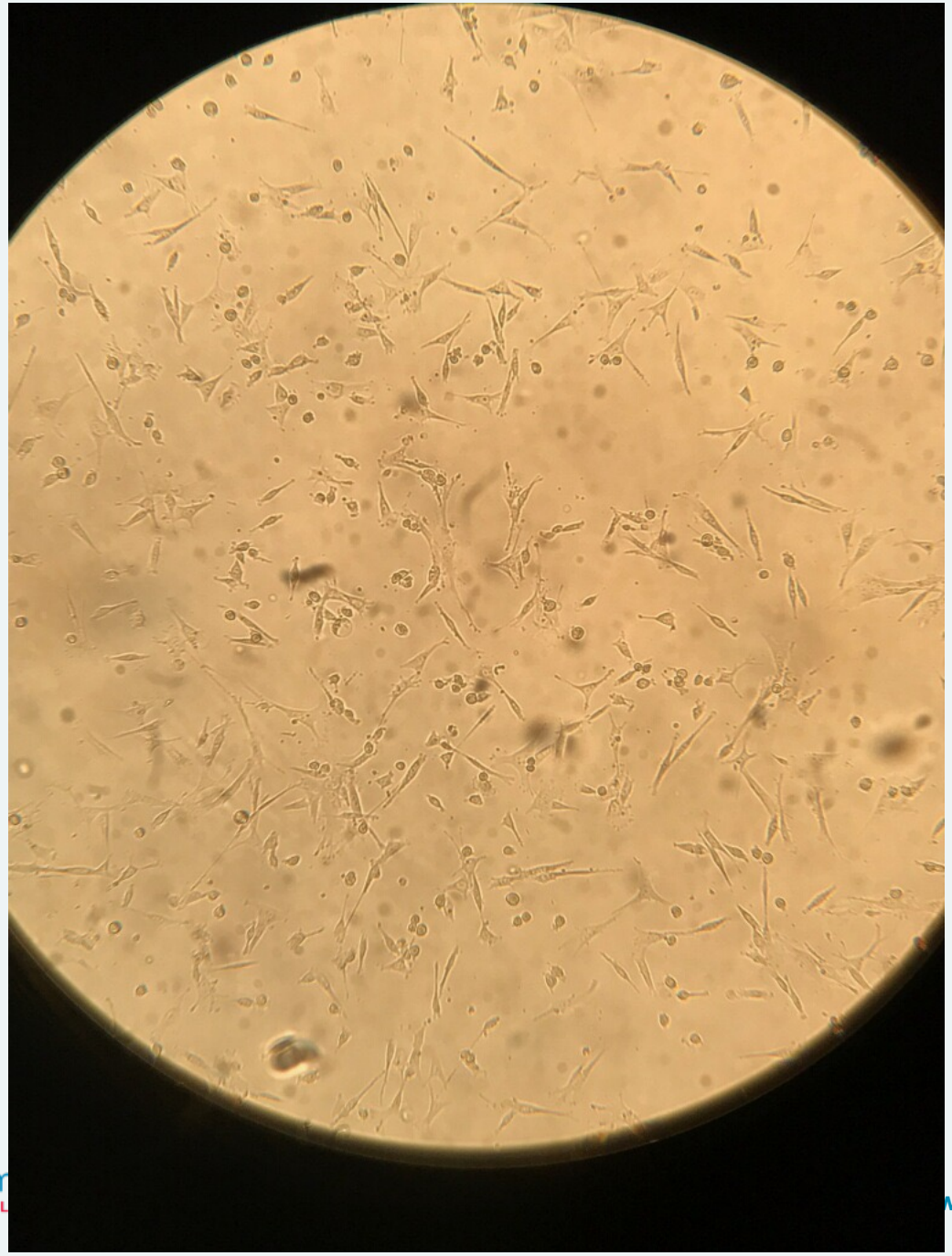

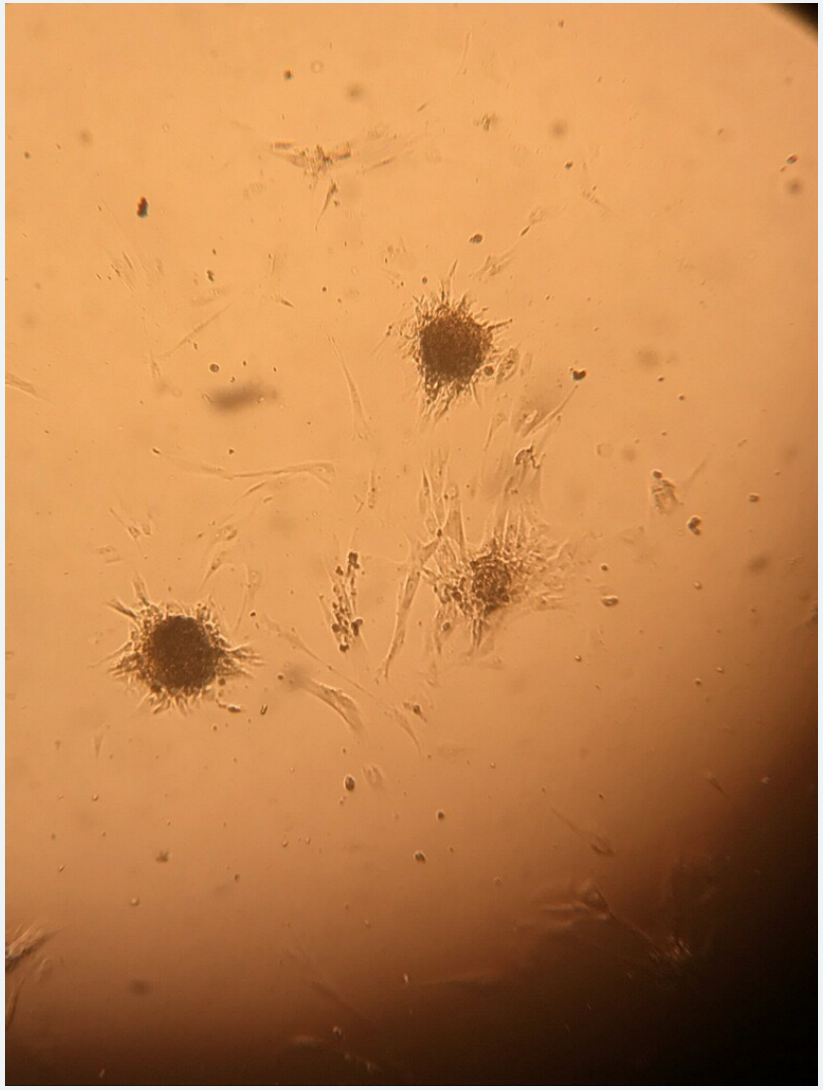

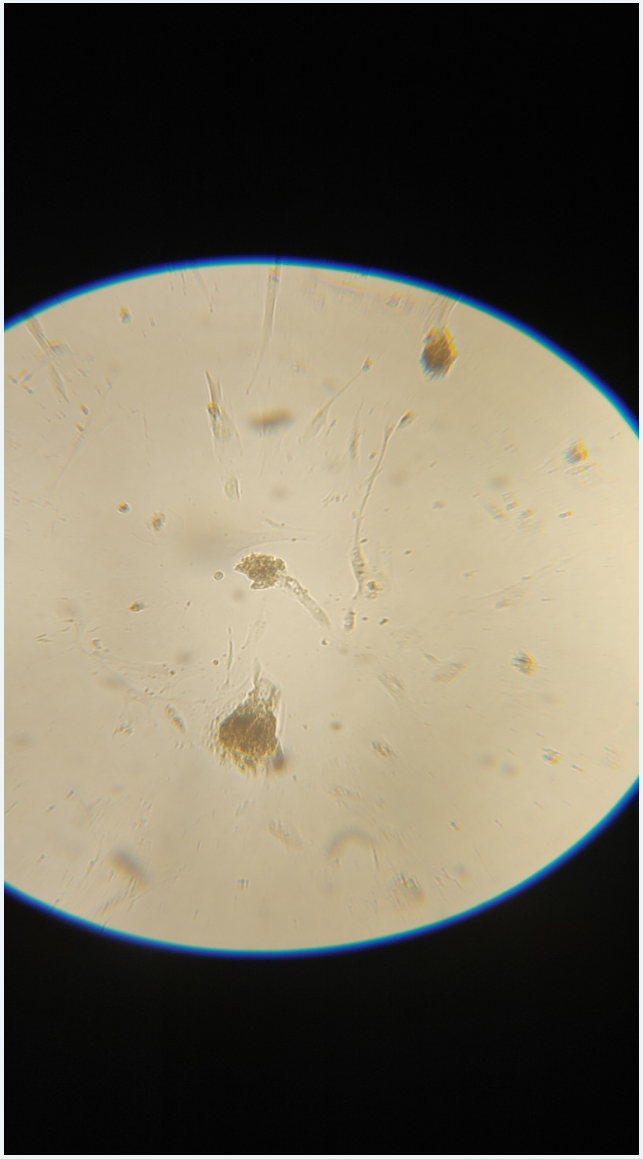

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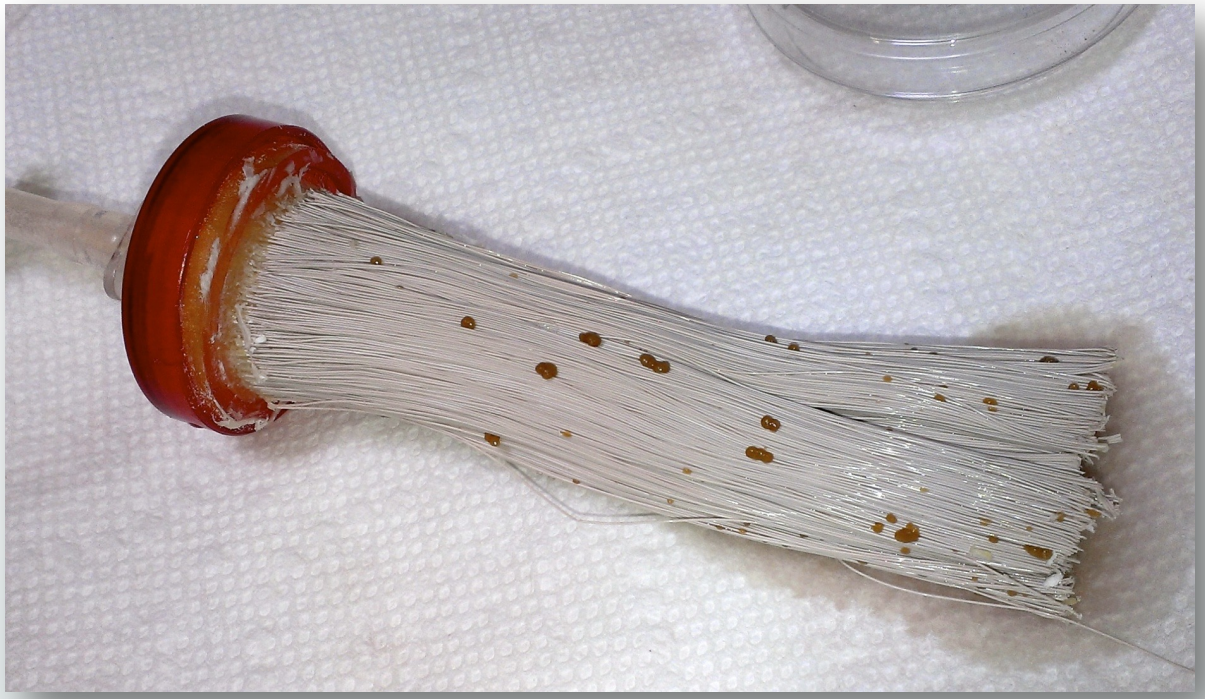
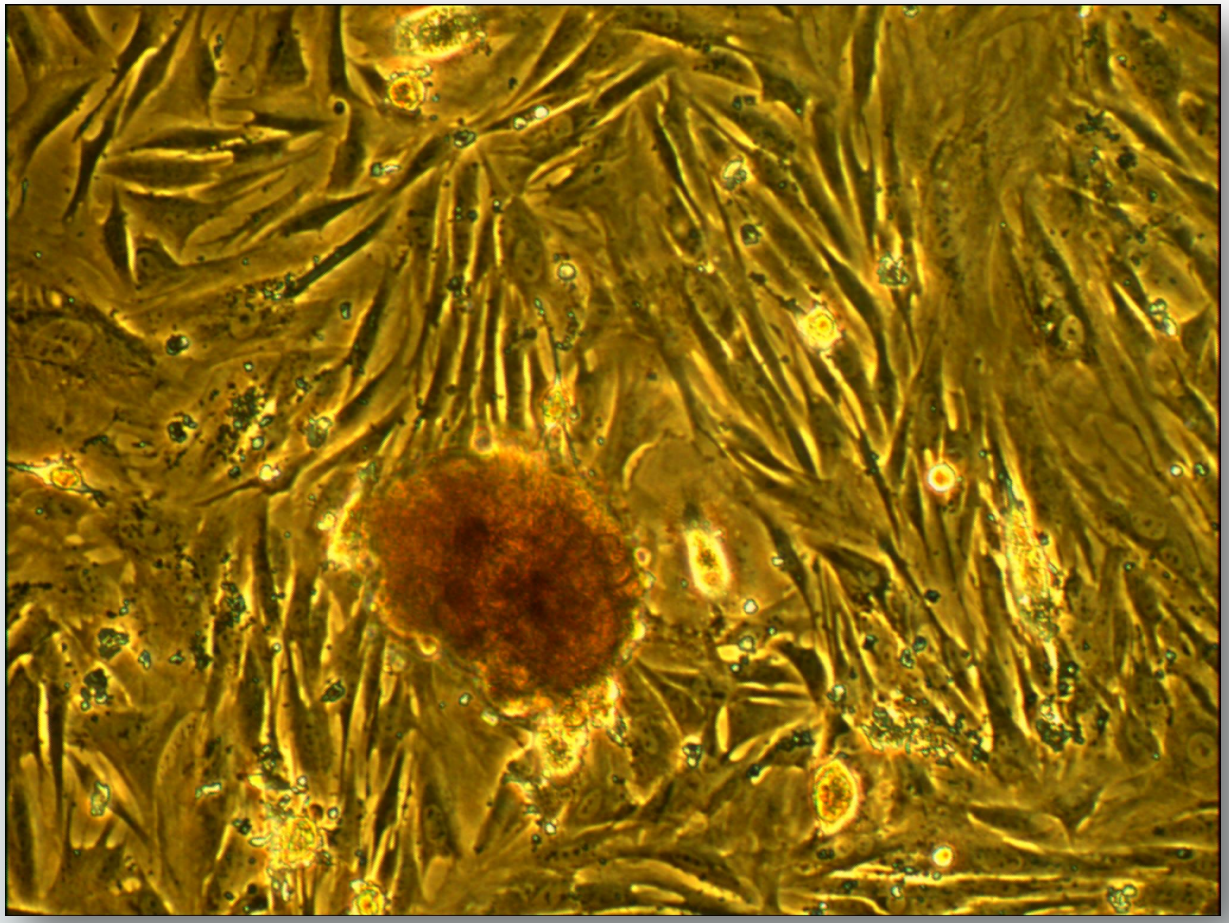
Pulsatile Perfusion of Placenta

Placental Co-Culture





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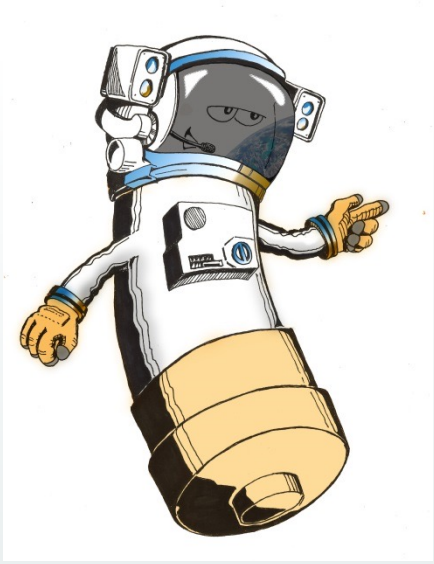
Harvest vs. Flask

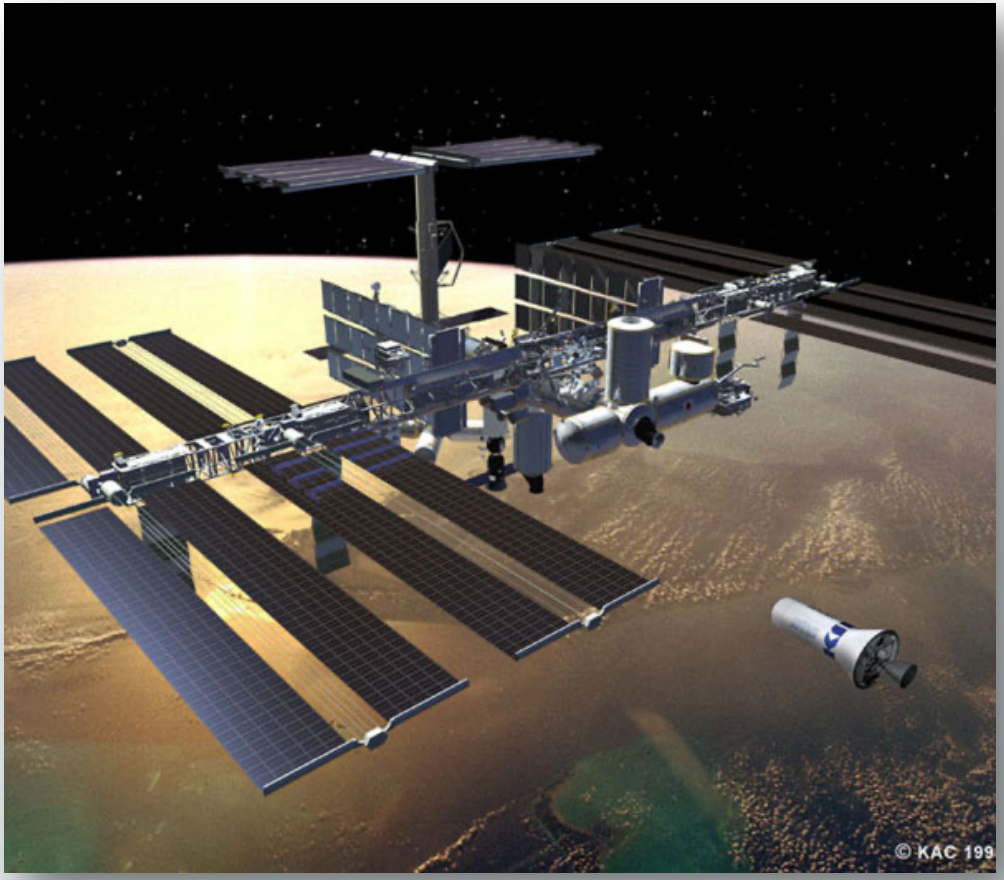
Phenotype	ECS Harvest	Flask
CD 45	4%	1%
CD 34	0%	0%
CD 133/2	2%	0%
CD 31	3%	48%
CD 13	6%	83%
CD 105	43%	99%
CD 73	18%	99%
CD 90	5%	96%
CD 14	23%	4%
NANOG	0%	0%

Summary

- The most *in vivo* method for culturing cells over long periods of time
- Hollow fiber was 3-D before the importance of 3-D culture was recognized
- Can be the only way to get two different cell types, in close enough proximity, at high enough density, for long enough time to observe interactions between the cells.
- 4-D culture, enough time for cells to self-organize and for these interactions to develop.

FiberCell Systems HFBR in Space





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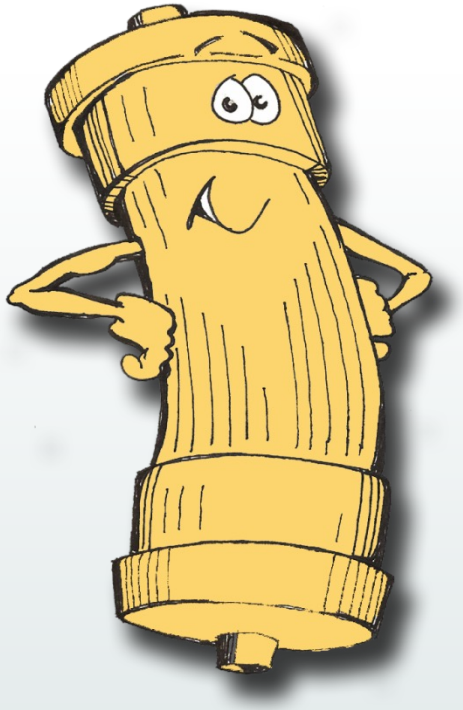
Thank you.



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