

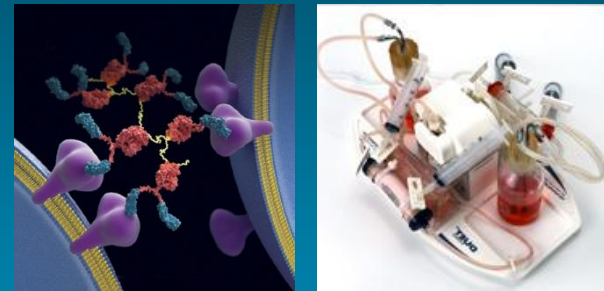
Research use only!  
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# Clinical Scale Production of Extracellular Vesicles in a Hollow Fiber Bioreactor

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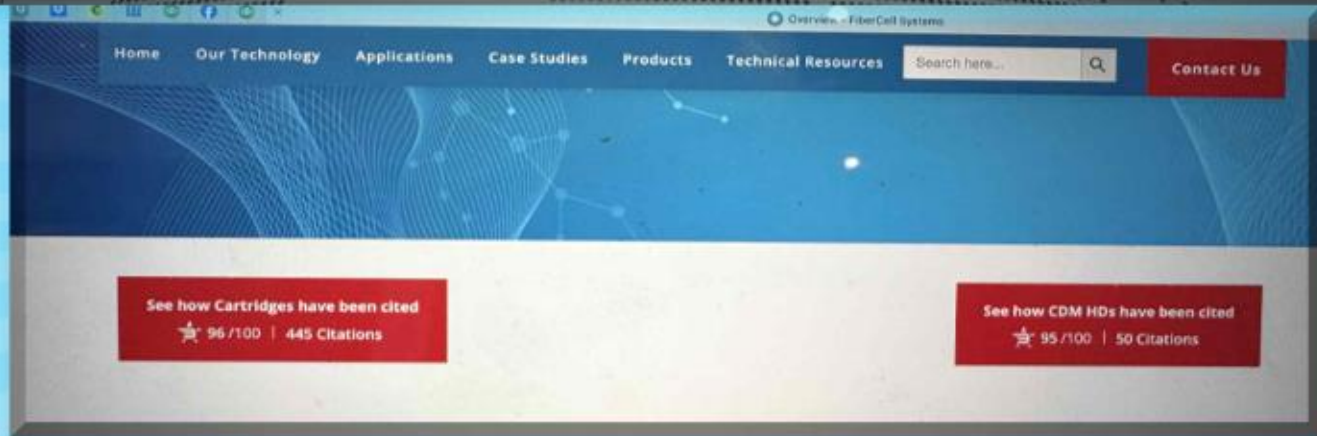
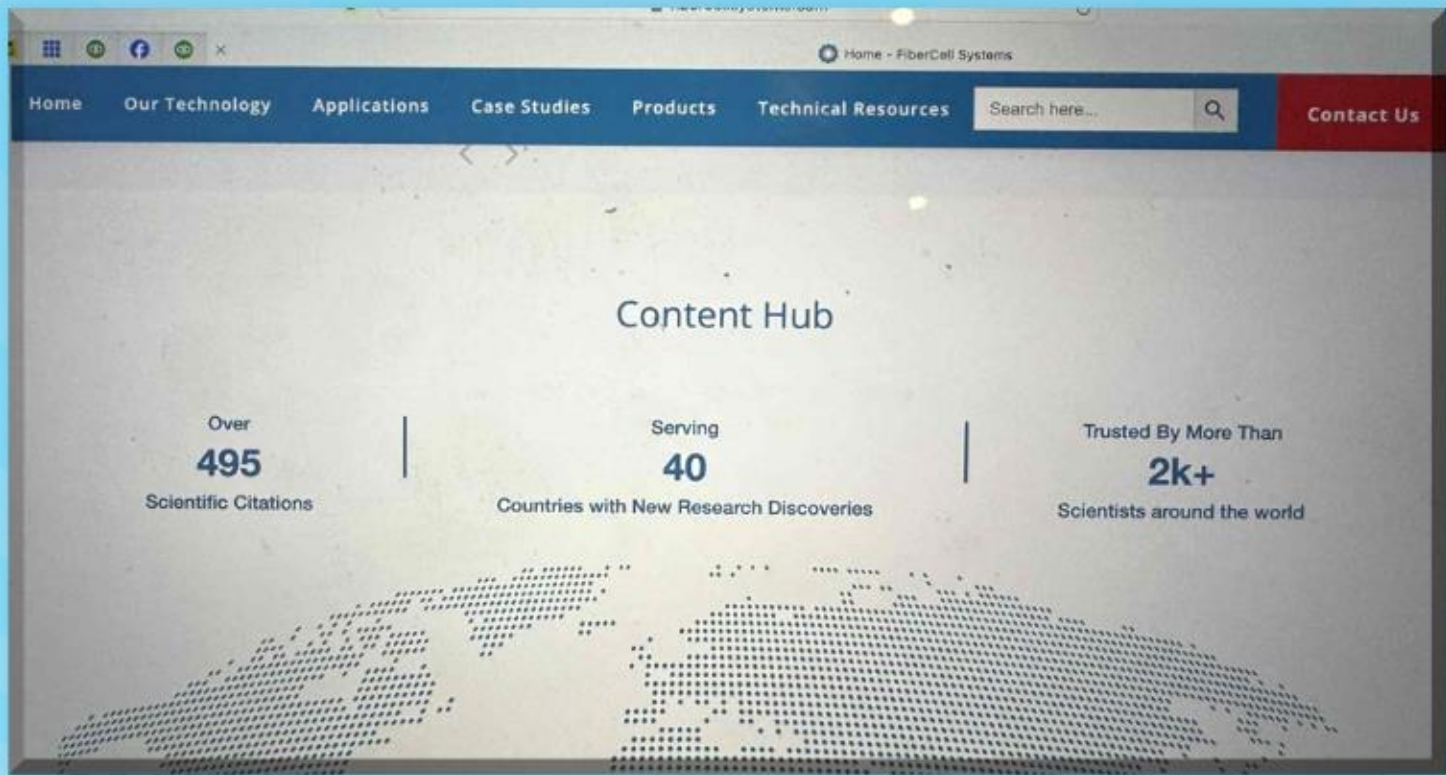
*By John J. S. Cadwell*



[www.fibercellsystems.com](http://www.fibercellsystems.com)

# About FiberCell Systems Inc.

- Founded in 2000 by John J.S. Cadwell, President & CEO
- Over **25 years** advancing hollow fiber bioreactor technology
  - Supported by **500+ peer-reviewed publications** from scientists worldwide
- Trusted leader in laboratory-scale hollow fiber bioreactors

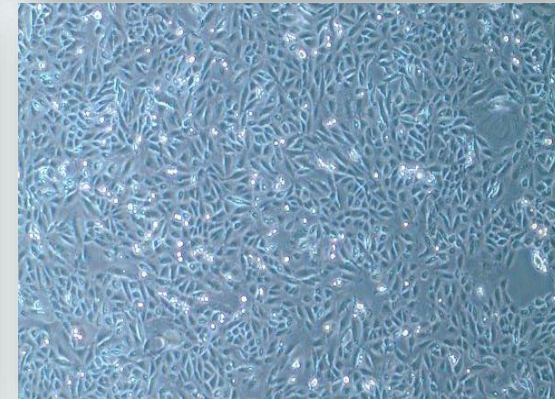
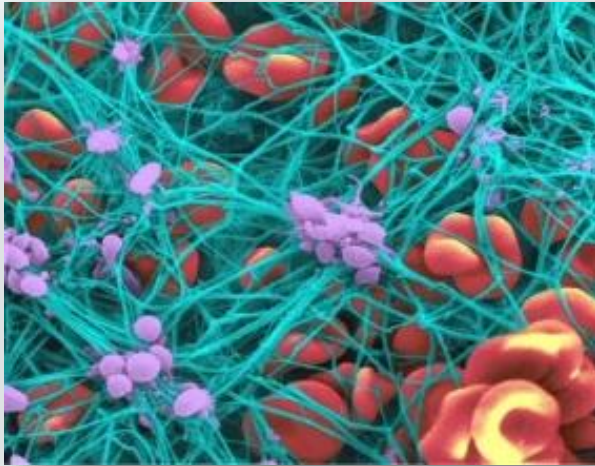


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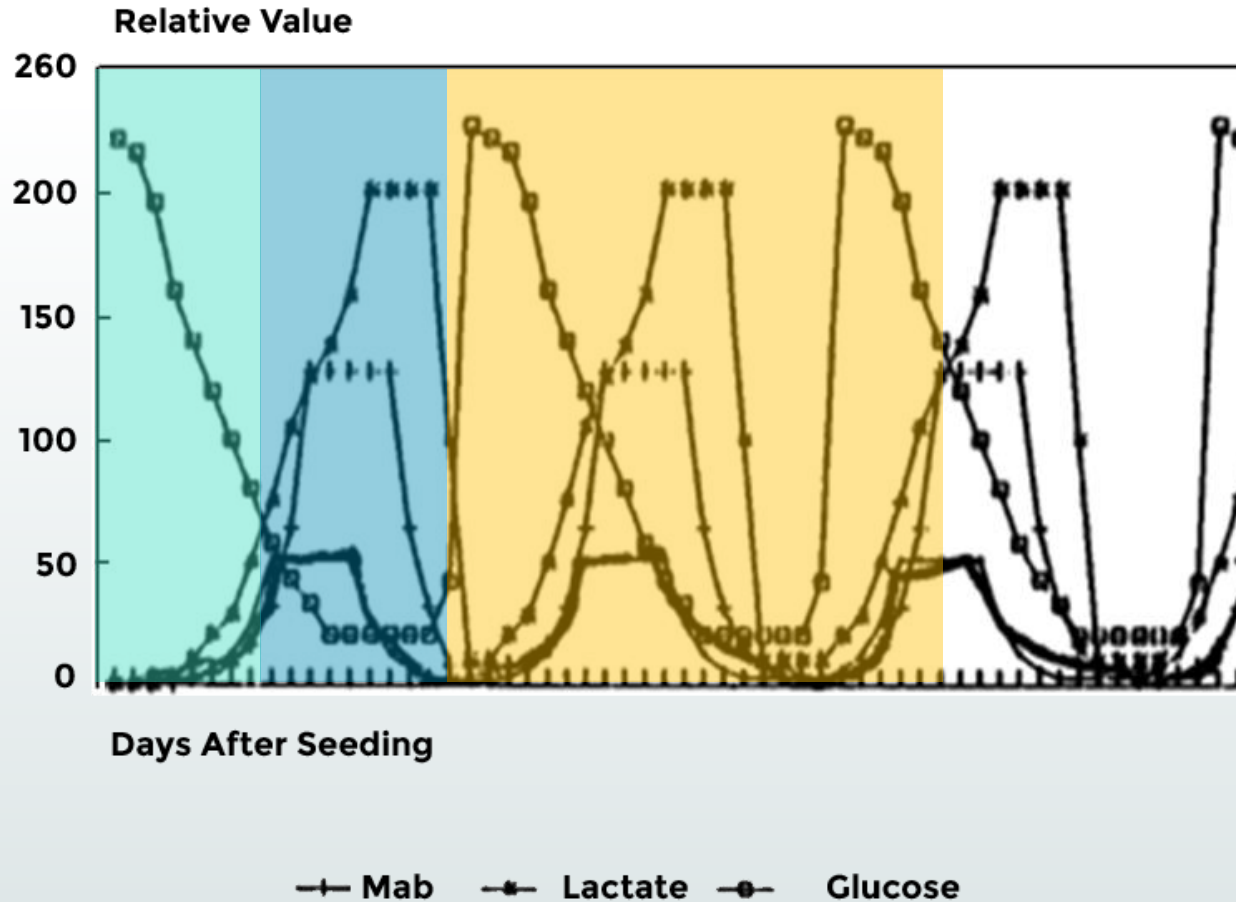
# Outline

- \* Historical Perspective
- \* Histocentric Principles of Hollow Fiber Bioreactors
- \* *In vivo*-like examples (MSC and HAFSC)
- \* EV Harvests: Do the impossible, see the unseeable
- \* Evolution and outer space.....

# Cell Culture Through the Ages



# “Feast or Famine”

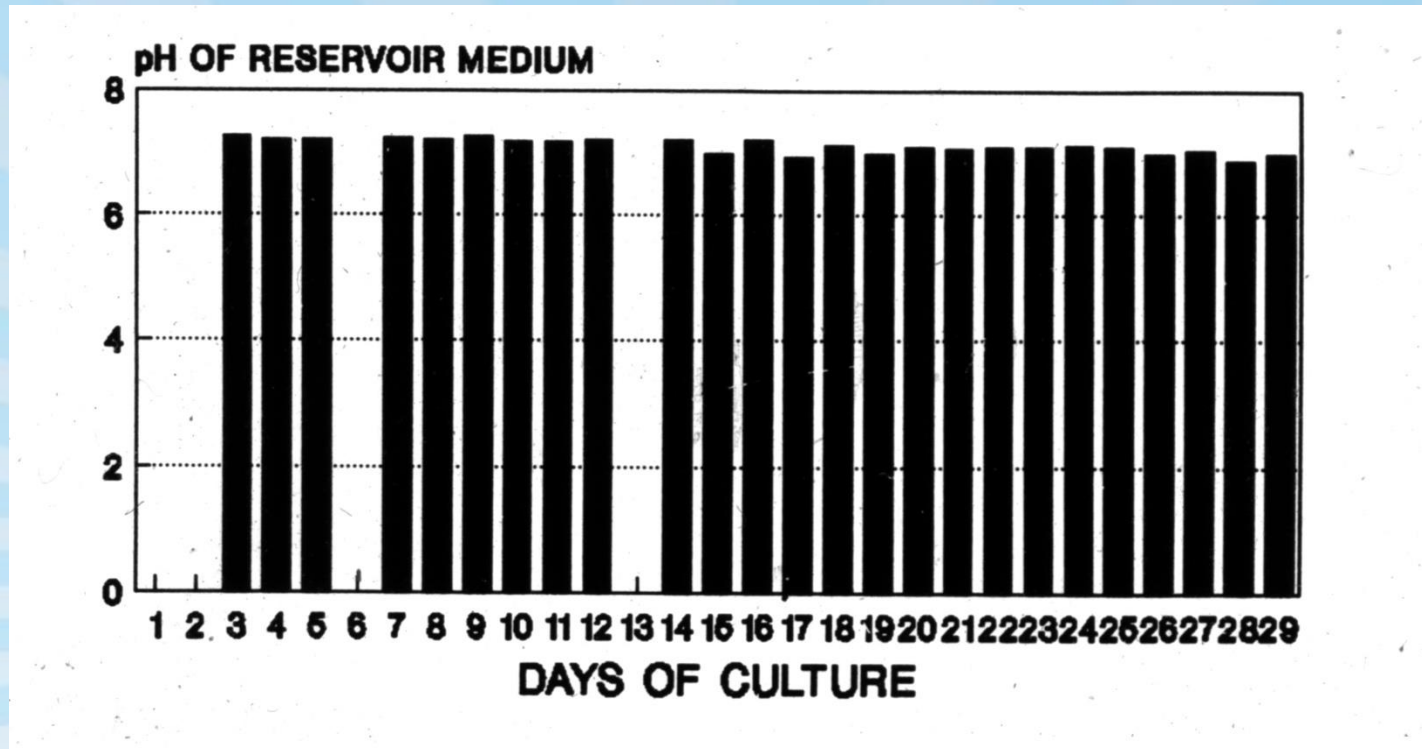


Not very physiologic!



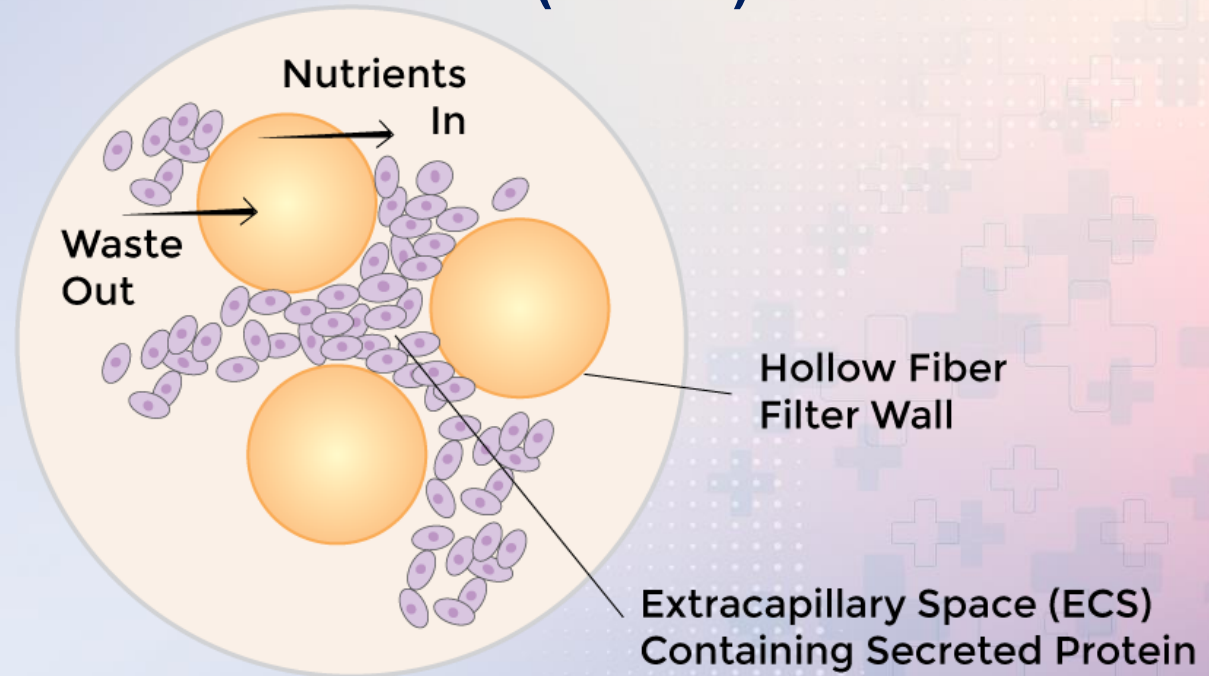
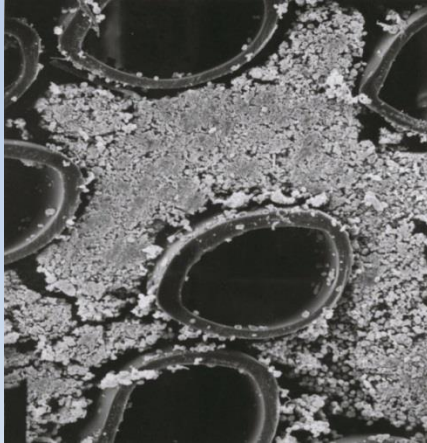
# Hollow Fiber Culture of CHO Cells, pH Changes

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Homeostasis

# What is a Hollow Fiber Bioreactor (HFBR)?



## A 3-D Bioreactor for High-Density Cell Culture

**Composition:** Thousands of porous, semi-permeable capillary fibers in a cartridge.

**Cell Residence:** Cells grow in the *extracapillary space (ECS)*, bound to the porous fibers.

**Media Flow:** Culture medium is pumped through the *lumen* of the fibers.

**Exchange:** Nutrients diffuse out; waste products diffuse in.

**Product Retention:** Secreted products (proteins, exosomes) are concentrated in the ECS.

*Hollow fiber was 3-D  
before 3-D was cool!*

# Principles of Histocentric Hollow Fiber Bioreactors

Histocentric Bioreactors recreate the *in vivo* microenvironment while allowing cell physiology to develop over time. 2-D, 3-D and now 4-D cell culture.

- Biological Mimicry
- Controlled Environment
- Dynamic Culture
- Cell Derived Microenvironment
- Compatibility With Various Cell Types
- Long Term Culture

# Key Advantages of Histocentric HFBR

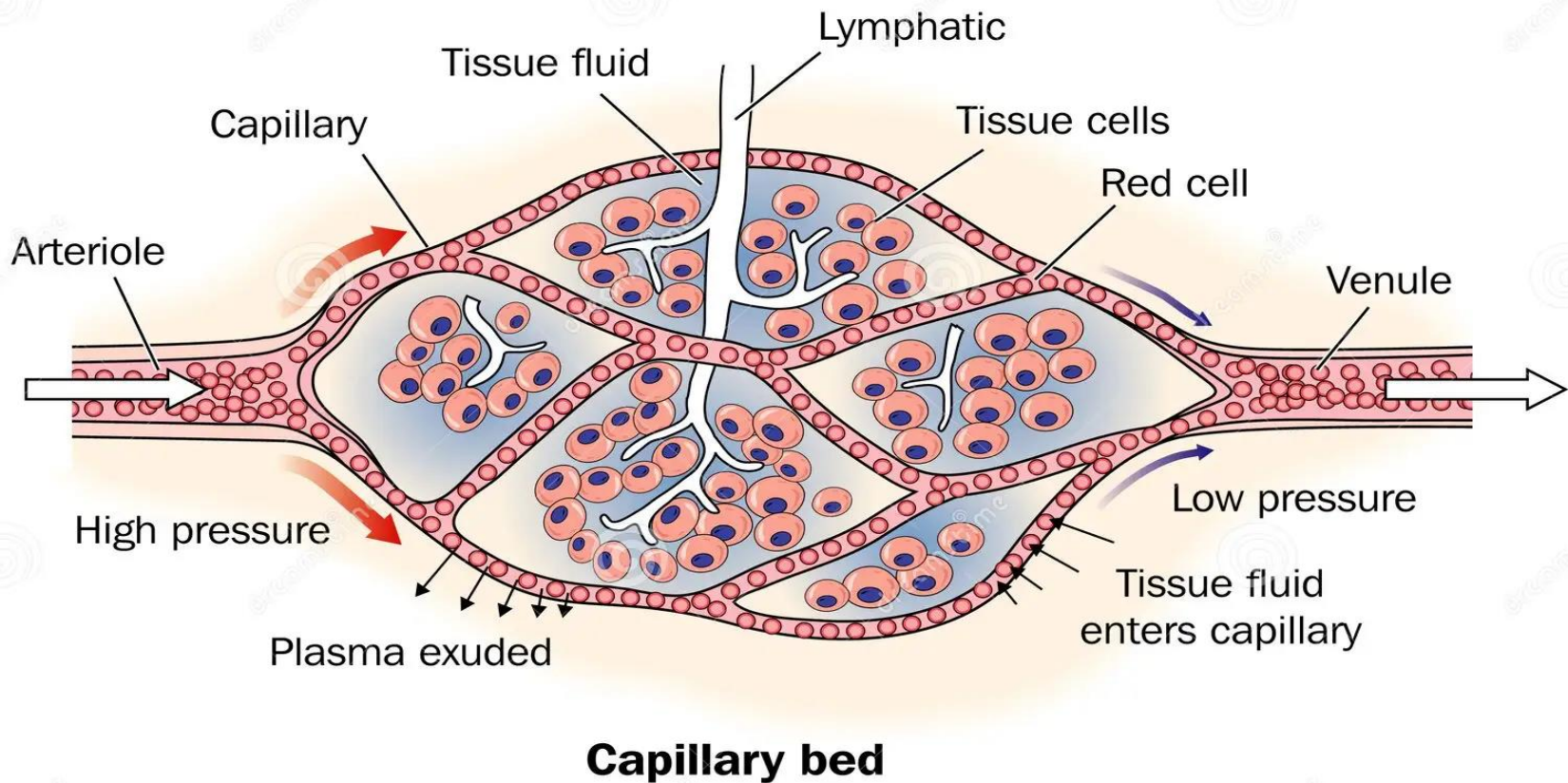
- Extremely high surface area/volume permits high density cell culture.
- Cells are bound to a porous support, not a non-porous 2-D flask.
- The molecular weight cut off (MWCO) of the fibers retains and concentrates secreted products.



# Illustration of how cells grows *in vivo* compared to HFBR

Histocentric Bioreactor attempts to recapitulate the *in-vivo* microenvironment.

Q: How many different medias are there in the human body?



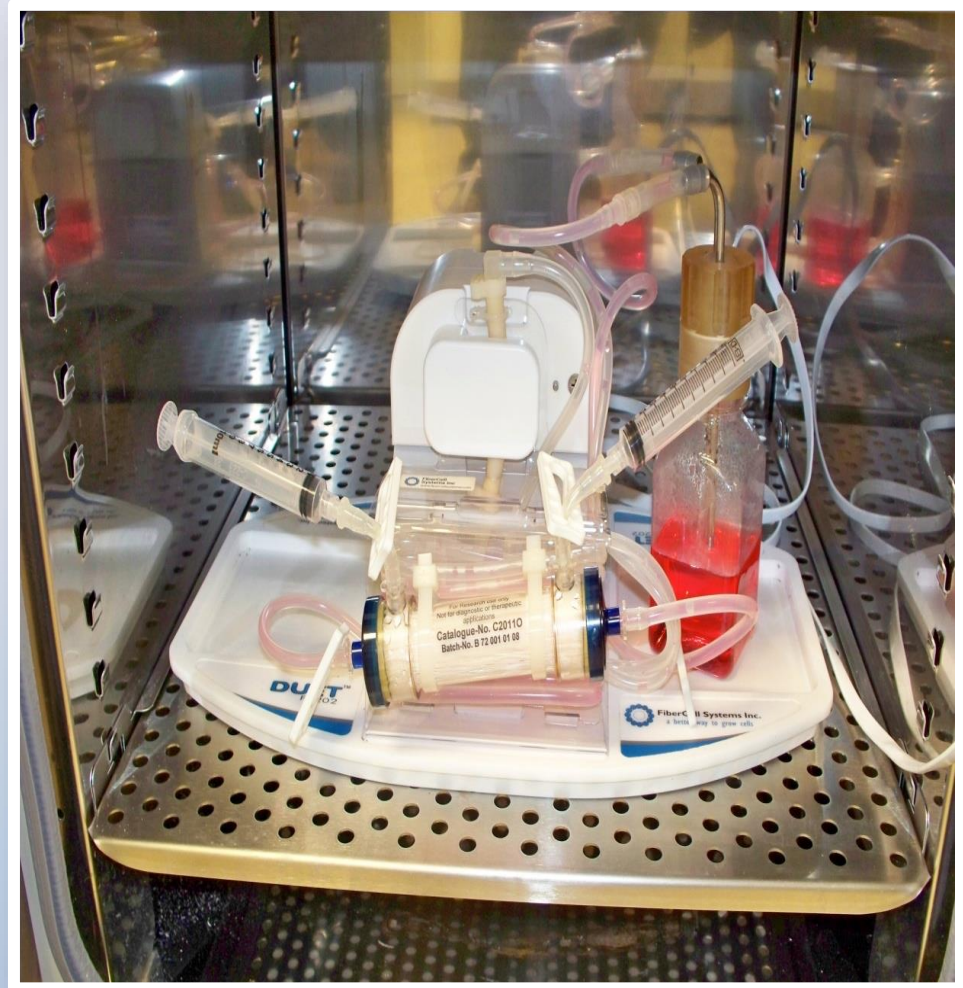
# Illustration of how cell grow at high density



*Best for collecting secreted products!*

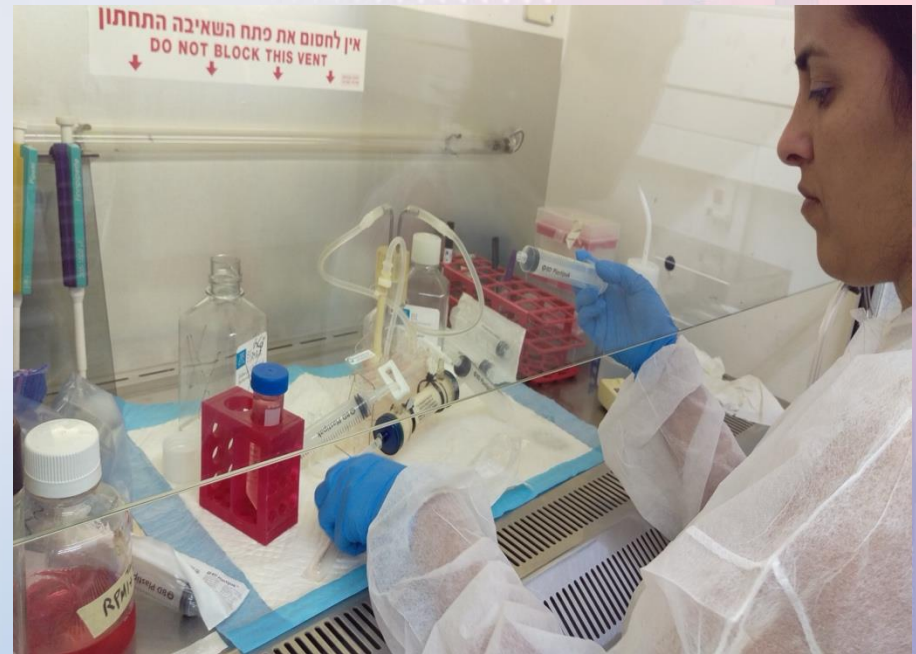
# HFBR In the Laboratory

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



# Working with HFBR In the Laboratory

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



# Advantages of Hollow Fiber Cell Culture

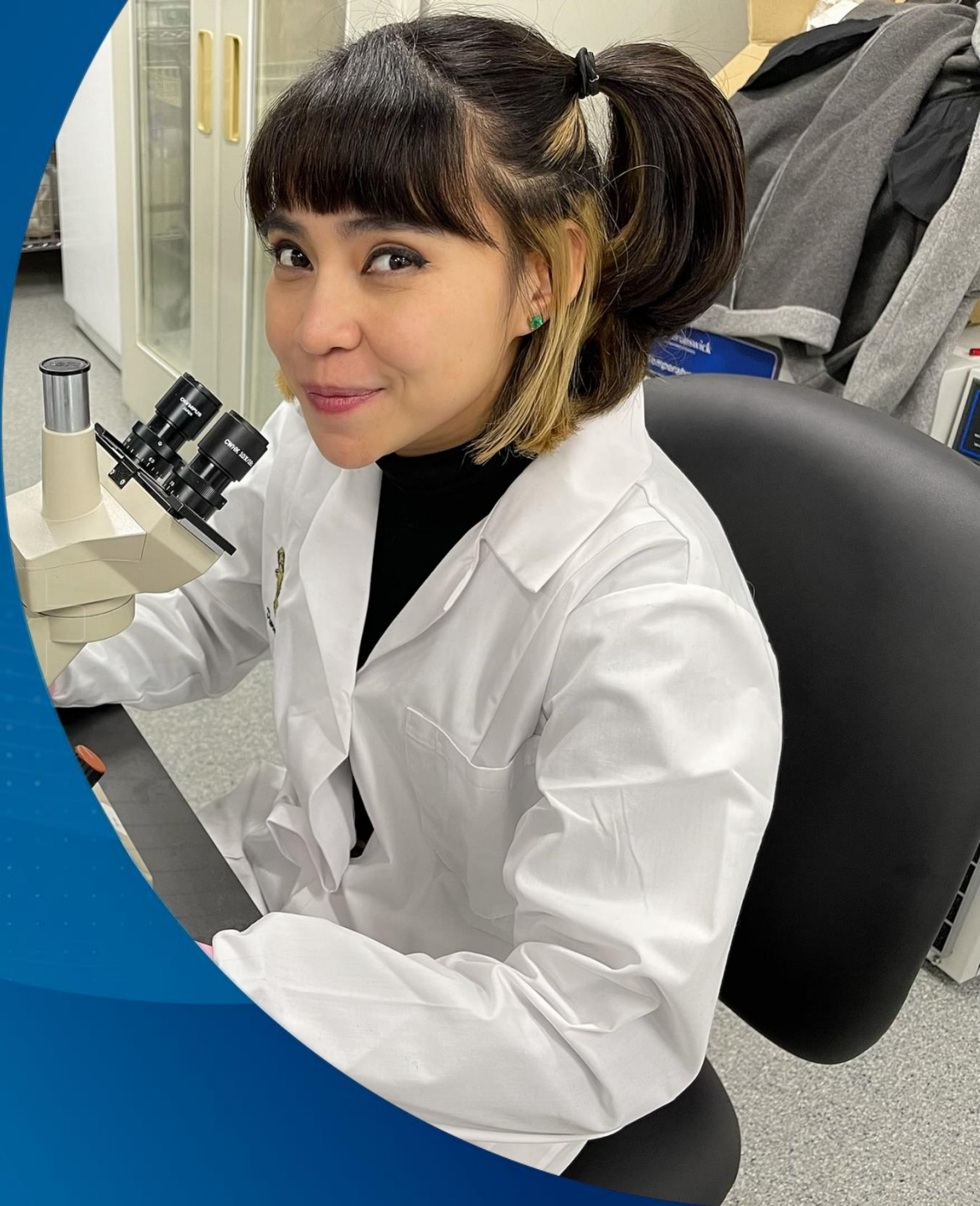
- Concentrated product
- Uniform and complete post-translational modifications
- Low apoptosis, less contamination with intracellular proteins and DNA
- Protein free medium (CDM-HD) contains no surfactants
- Consistency of production over many months



# Plastic Waste Generated by $1 \times 10^9$ Cells

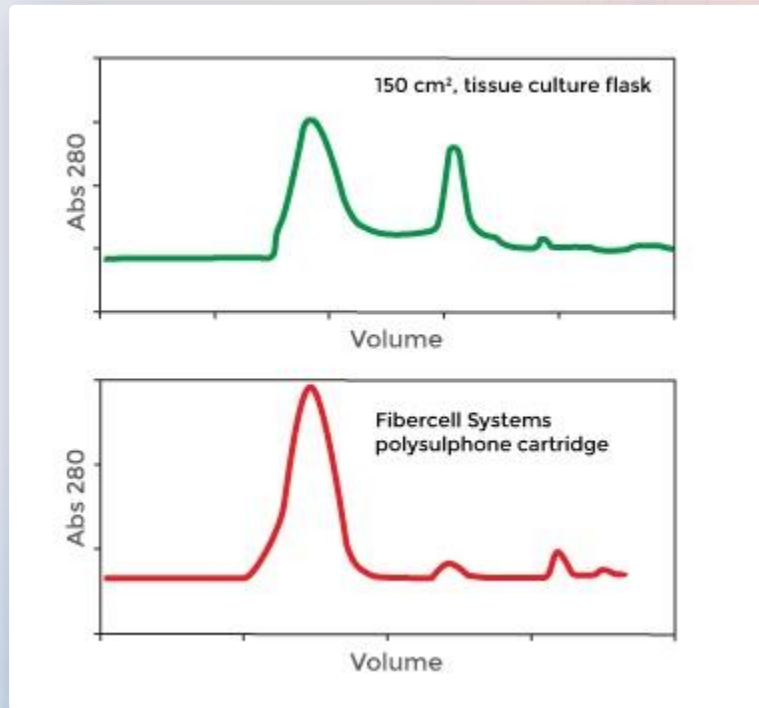


3-D  
Examples...



# Recombinant Protein Production

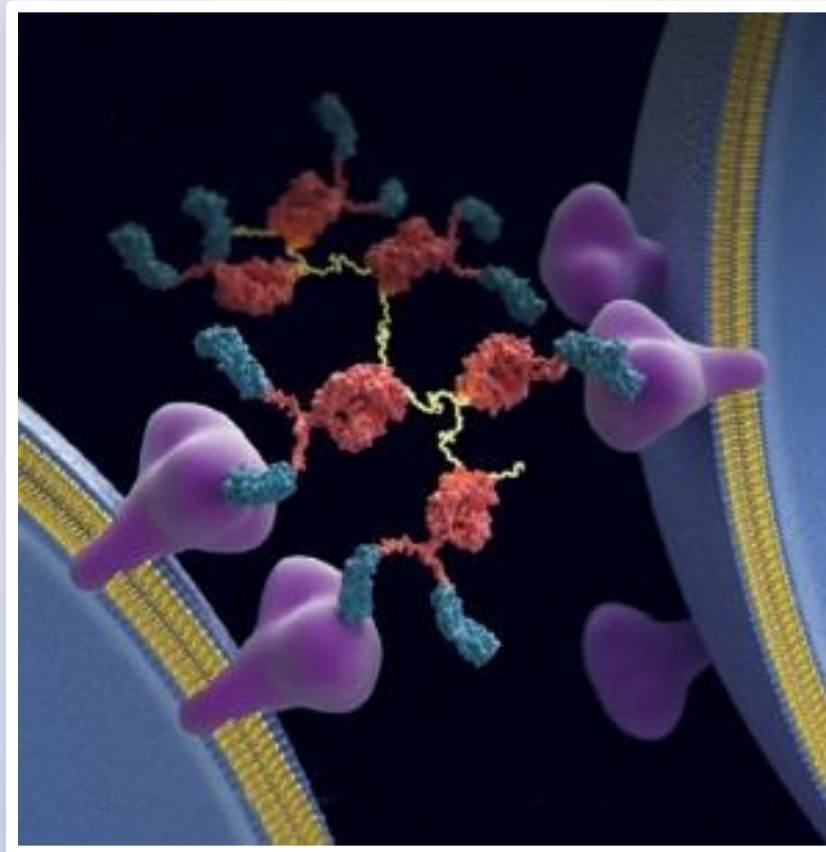
- + Both suspension and adherent cell types
- + 100x+ higher concentration
- + Easily adapt to SFM
- + Can provide improved protein folding



# Journal of Biological Chemistry, Sept 2007

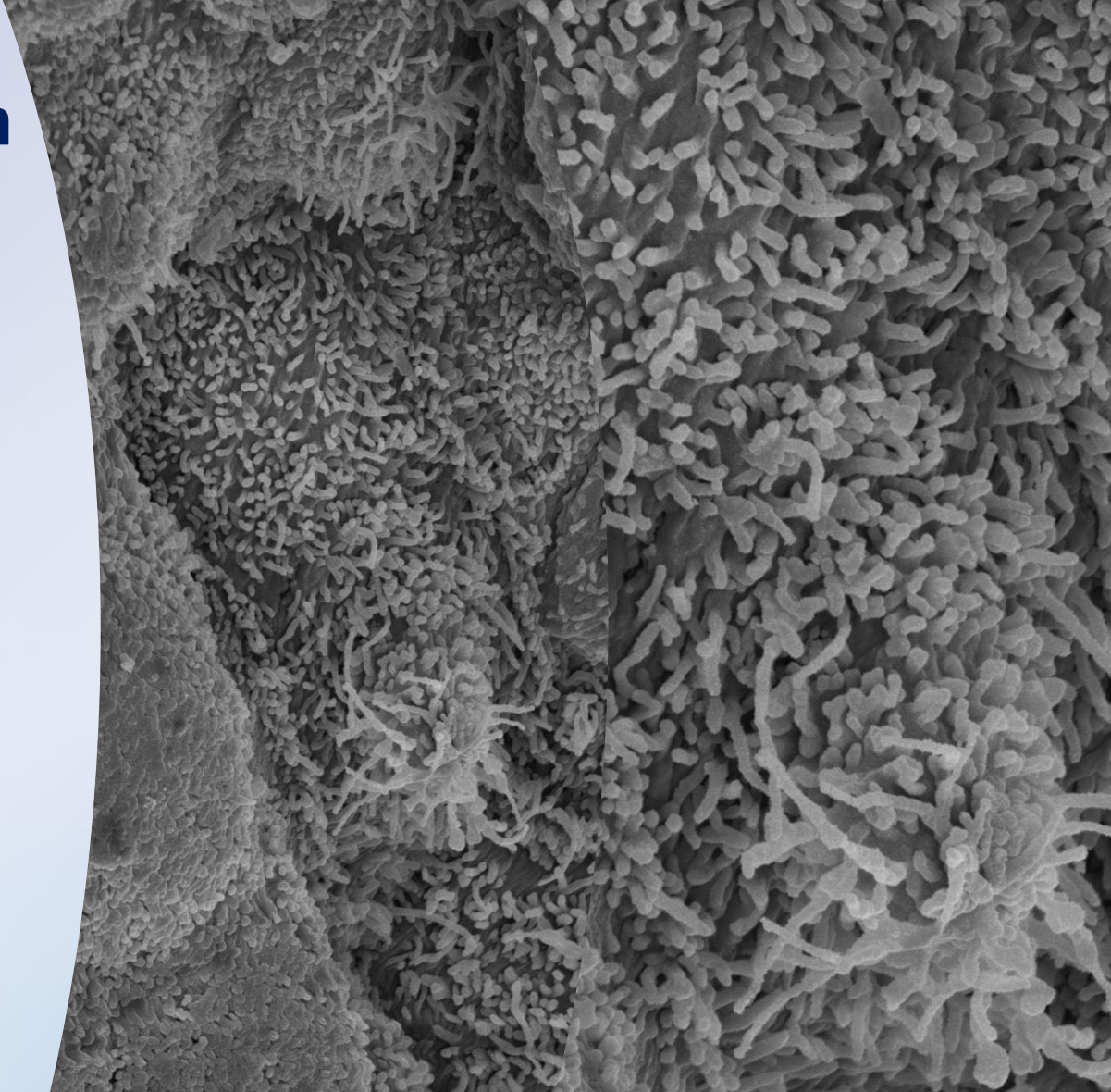
## Hexamerized IgG

6 IgG1 subunits held together with 3 IgA tails. Fv region substituted with CD4 receptor to cross-link HIV and inactivate it.



# Cryptosporidium

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



Albert Einstein College of Medicine  
OF YESHIVA UNIVERSITY

Mag = EIN

Reference Mag = Einstein College of Medicine  
UNIVERSITY

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Reference Mag = Polaroid 545 Date :6

# Primary lung tissue inoculated as a single cell suspension and supported by CDM HD.

Dr. Akhil Srivastava, U. Missouri



# Exosome Cell Culture Conditions Affect EV Composition

## Laminar Flow Alters EV Composition in HUVECs: A Study of Culture Medium Optimization and Molecular Profiling of Vesicle Cargo

*Arefeh Kardani, Jan Hemmer, Britta Diesel, Vida Mashayekhi, Annika Schomisch, Marcus Koch, Claudia Fecher-Trost, Markus R Meyer, Nicole Ludwig, Shusruto Rishik, Andreas Keller, Jessica Hoppstädter, Gregor Fuhrmann,\* and Alexandra K. Kiemer\**

Endothelial cells (ECs) experience shear stress associated with blood flow. Such shear stress regulates endothelial function by altering cell physiology. Since most cell culture protocols and media compositions are designed for static cultures and experiments with ECs are predominantly conducted under these non-physiological conditions, a model for culturing ECs under flow conditions is developed, which more closely mimics their physiological environment. This approach also enables the isolation of EVs while minimizing FCS-derived contaminants. In this study, a comprehensive assessment of how physiologically relevant cultivation conditions influence the vesicle composition and function of ECs is provided. A detailed investigation is conducted for the effect of different cell culture media on morphology and marker expression of human umbilical cord endothelial cells (HUVECs) and EVs, and optimize the conditions to culture ECs under flow, tailoring them specifically to facilitate the efficient isolation of EVs using a hollow-fiber system model. These EVs are then characterized and compared to those isolated from traditional static culture conditions. Overall, this study presents a model on isolating EC-derived EVs under conditions that closely mimic physiological environments, and characterization at their proteome, gene expression, and microRNA profile.

### 1. Introduction

Extracellular vesicles (EVs) are nanosized membrane-bound structures released by almost all types of cells into their external environment. Eukaryotic EVs are usually classified into three main categories, based on their size and mode of production.<sup>[1]</sup> Microvesicles are formed by the outward budding of membrane vesicles from the cell surface.<sup>[2]</sup> Exosomes originate from the endocytic pathway through the 'outward' budding of the late endosomal membrane. Initially, they accumulate in structures known as multivesicular bodies (MVBs), which later fuse with the plasma membrane, releasing their contents as exosomes into the extracellular space.<sup>[3]</sup> The third major type of eukaryotic EVs called apoptotic bodies are produced from cells undergoing programmed cell death by outward budding from the surface of apoptotic cell.<sup>[4]</sup>

# Pulsatile Perfusion of Placenta



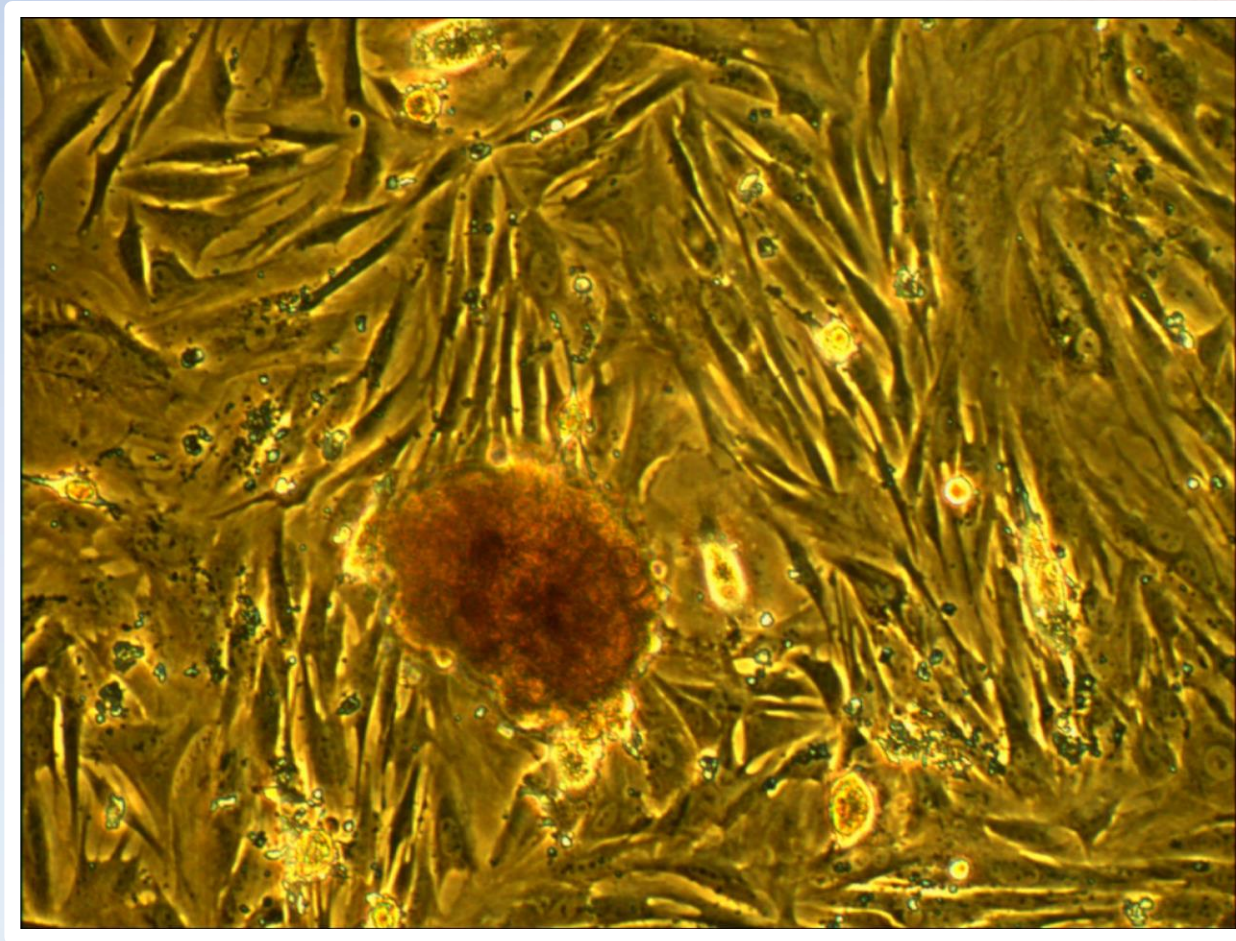
# Placental Co-Culture



# 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%

# Cells Harvested from HFBR & Placed in a Flask

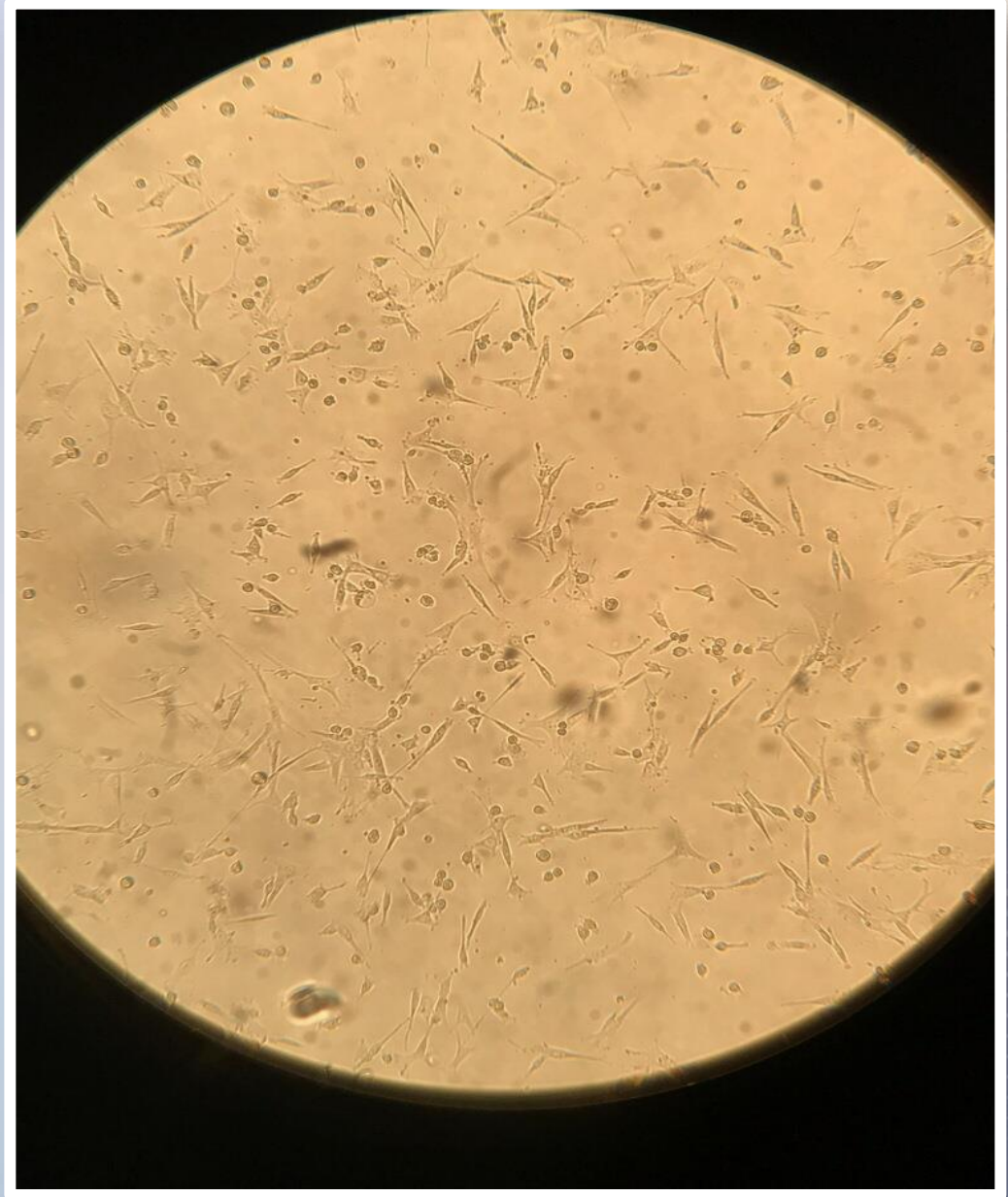


## What are MSC?

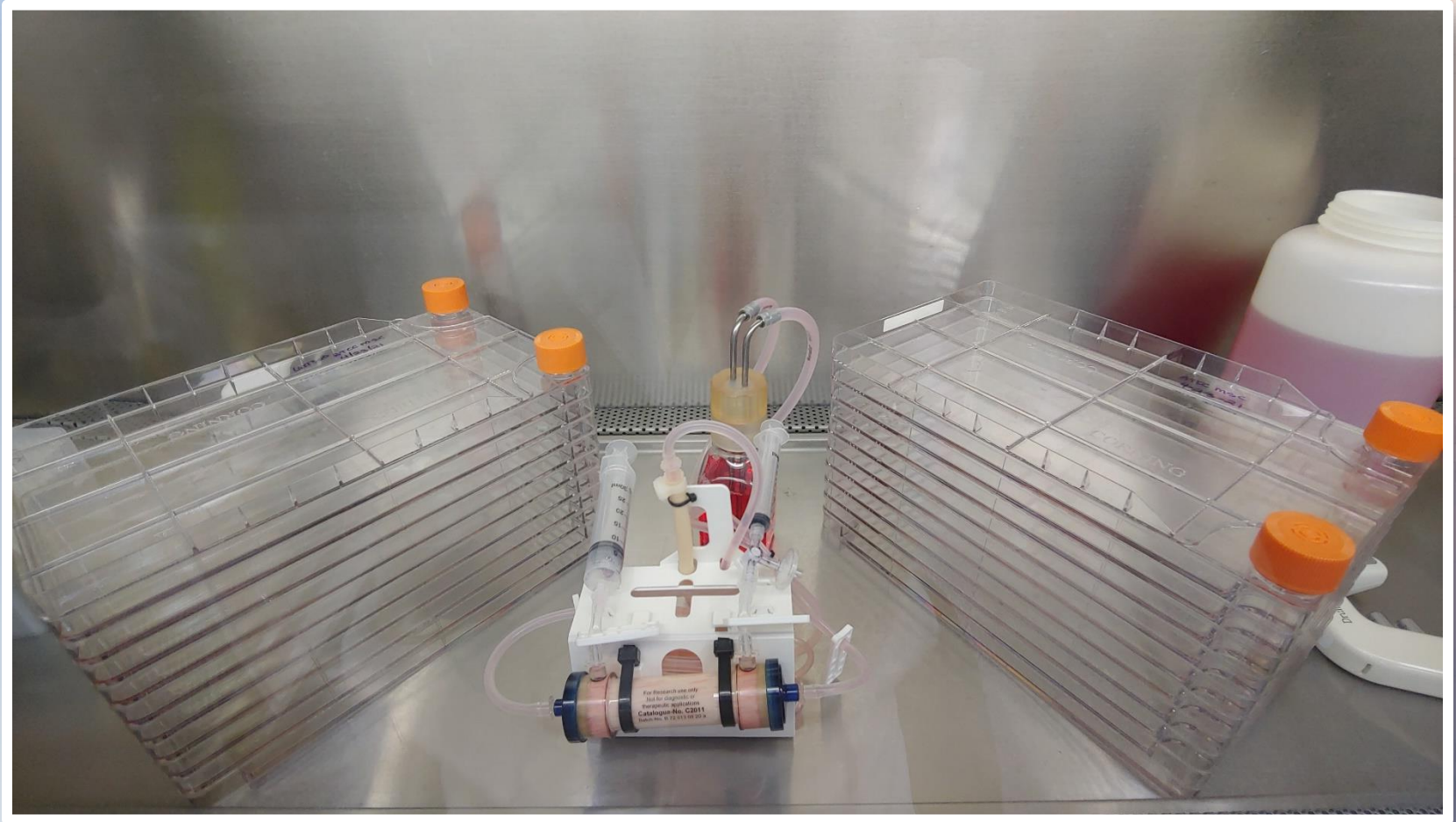
MSCs are plastic adherent fibroblastic cells with the “trilineage potential” of osteogenic, chondrogenic and adipogenic differentiation capabilities. Furthermore, they express the cell surface markers CD73, CD90, and CD105, and do not express haematopoietic and endothelial antigens (CD14 or CD11b, CD19 or CD79 $\alpha$ , 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

# MSC in 2-D Culture



# MSC in 3-D HFBR

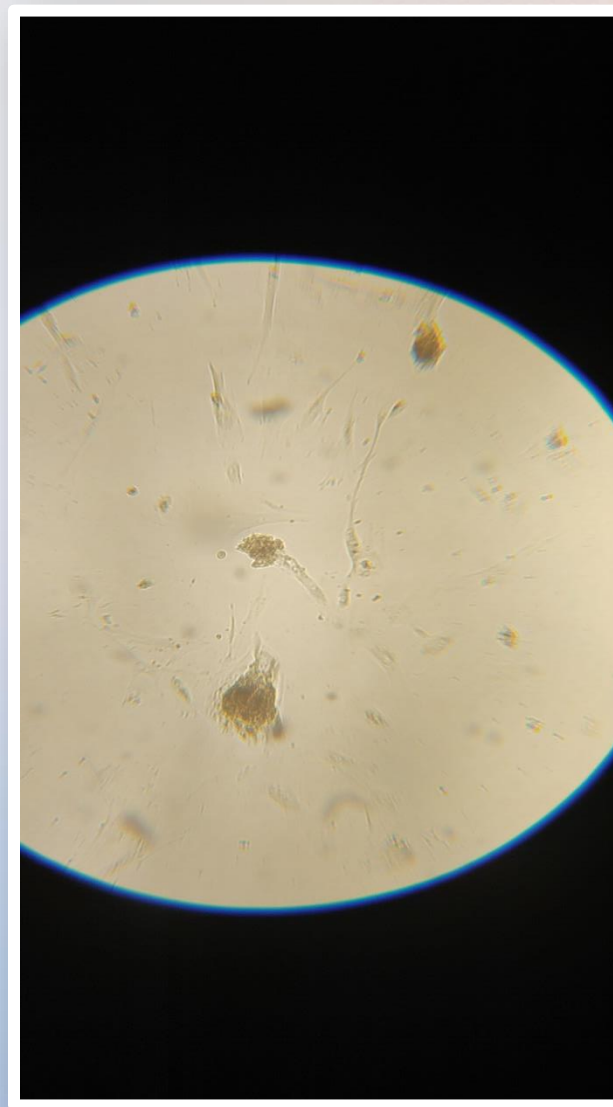
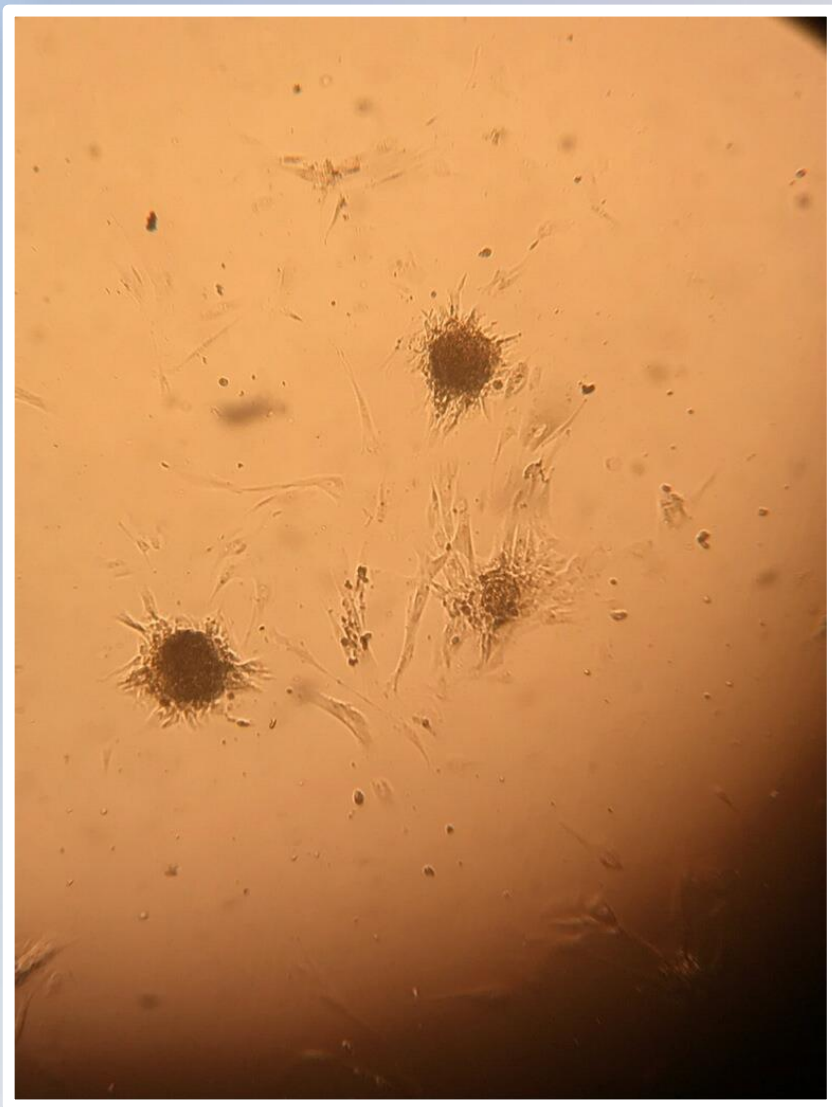


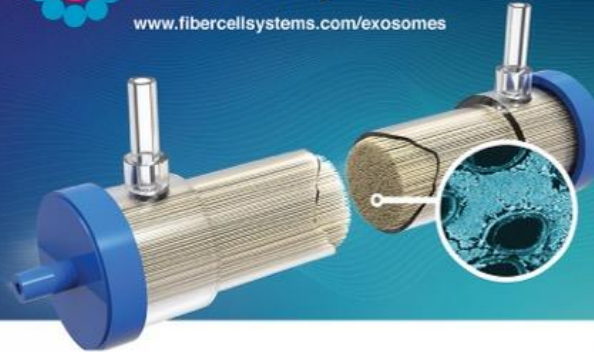
MSC do not proliferate in the 3-D HFBR

# Showing formation of Spheroids



# MSC in 2-D After 3-D





**Retroviral Transduction and Production of Palm-GRET Labelled Extracellular Vesicles using Bone Marrow Derived MSC in a Hollow Fiber Bioreactor.**

John J.S. Cadwell (1), Samantha S. Dostalk (1), Blanca Rodriguez (2), Lilia Panushkina (2), Zhaochao Liao (2), and Ken Witwer (2)

1. FiberCell Systems Inc., Frederick, Md., USA
2. Johns Hopkins Medical School, Baltimore, USA

**Introduction**

Various methods have been applied to the transfer of genes into mesenchymal stem cells (MSC). The generation of stable MSC transfectants is hampered by the limited number of passages MSC can undergo before they start to differentiate and difficulty in performing at clinical scale. Current data suggests that mesenchymal stem cells, when seeded into a 3-D hollow fiber bioreactor (HFBR) show little proliferation and may be maintained in culture and produce EVs continuously for extended periods of time at high concentrations. Transient transfection under these conditions could result in usefully stable MSC transfectants. To this end a retroviral transfection of bone marrow MSC using an HFBR was performed.

**Methods**

Bone marrow MSC from ATCC were expanded to  $5 \times 10^7$  cells using DMEM/10% FBS and 10 T300 flasks. Retrovirus encoding for green fluorescent protein and Nanoluciferase protein was produced in culture. A FiberCell Systems C202SD 20 kD MWCO polysulfone cartridge with 450 cm<sup>2</sup> of area and a 2.8 ml. volume was seeded as follows.  $5 \times 10^7$  cells in a volume of 5 ml. was attached to one side-port and  $4.5 \times 10^8$  retrovirus in a volume of 4 ml. was attached to the opposite side port. Equal volume of cell/virus mixture was flushed into each syringe and then injected into the ECS of the cartridge with the excess volume flowing through the fibers into the medium reservoir, concentrating the cells and the virus together. After 24 hours the ECS was drained, and the medium replaced with basal DMEM (no serum). Harvests were performed at 24-hour intervals for four weeks.

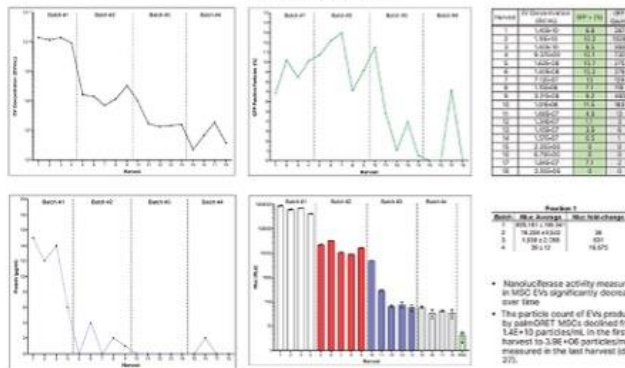
**Results**

1. Isolated EVs from the first 16 days of harvest collection show strong Nuc signal and approximately 5-12% of all detected particles in those EV samples were GFP positive, indicating release of the GFP-Nanoluc fusion reporter proteins via EVs by MSC transfectants.
2. The particle counts of EVs produced by palmGRET MSC declined from  $1.4 \times 10^{10}$  particles/mL in the first harvest to  $3.9 \times 10^9$  particles/mL measured in the last harvest (day 27).
3. EV production was drastically reduced after 2 weeks of MSC transduction in HFBR.
3. It was not really possible to directly determine transduction efficiency under these conditions.

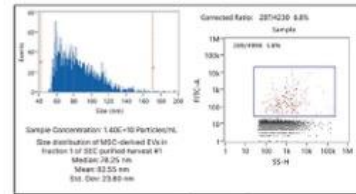
**Discussion**

A hollow fiber bioreactor can reduce the volume required to perform transductions by 100X, and utilizes a closed, cGMP compatible format. Overall, these promising preliminary data warrant further optimization and refinement of the transduction protocol, particularly by modifying the viral titer, selection strategy, and length of the experiment. Cell viability assays will also be performed to determine whether EV concentration declined after 14 days as a result of decreased cell viability or if other factors are at play. Increasing scale by a factor of 100x or more is possible using existing hollow fiber systems.

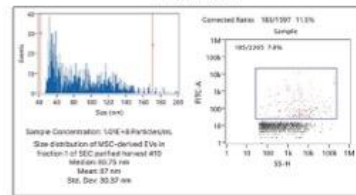
**Characterization of MSC EVs**



**Harvest #1 (02.08.2022) EVs (Fraction 1)**



**Harvest #1 (02.08.2022) EVs (Fraction 1)**



Here we determined the # of particles and the % of GFP+ particles in fraction 1 of harvest #1 of MSCs using Nanoluciferase

# Scalability of Production and Bio-Activity of Amniotic Fluid Stem Cell Extracellular Vesicles from 3-D Hollow Fiber Bioreactor and 2-D Culture.



**FiberCell Systems**  
www.fibercellsystems.com/en/060005

C Doshia (1), P Neviani (1), JJS Cadwell (2), S Dostalik (2), S Sedrakyan (1), L Perin (1)  
1) USC-Children's Hospital Los Angeles, USA  
2) FiberCell Systems, Frederick, Md, USA



## Introduction

EV clinical translation is constrained by limitations in scale-up of EVs production. Hollow fiber bioreactors (HFBR) support the culture of large numbers of cells, at high densities, producing significant numbers of EVs at high concentration. The high cell densities present in a HFBR can facilitate the use of xeno-free/chemically defined mediums, such as CDM-HD. Here we compare production, potency, identity and therapeutic potential of EVs collected from cells grown in culture dishes (2-D) vs. a HFBR (3-D).

## Methods

Human clonal Amniotic Fluid Stem Cells, hAFSC, were derived from consented donor's amniotic fluid.  $1 \times 10^6$  hAFSC were seeded in 2-D petri dishes (145 cm<sup>2</sup>) and 3,200 hAFSC were seeded into a 20 HD MWCO HFBR (FiberCell Systems C2011, 20 KD, 4,000 cm<sup>2</sup>) with fibronectin coating; both cultured in Chang's medium with 20% FBS. At confluence in the petri dish the medium was replaced with basal medium, stirred for 48 hr and EVs collected. After three days the medium in the ECS of the HFBR was replaced with Chang's medium alone, without 20% FBS, complete Chang's with 20% FBS remained in the central reservoir. The ECS was flushed with basal Chang's over the next 3 days and then harvesting of EVs every day was initiate. After two weeks of production serum in the reservoir was reduced stepwise to 5% and 5% CDM-HD introduced. After one more week serum was completely removed and replaced with 10% CDM-HD. The final weeks of EV production were produced using chemically defined medium, CDM-HD alone. Glucose consumption was monitored on a daily basis. 2-D EVs and 3-D EVs were compared by Nanosight, potency assay and by WB and therapeutic effect *in vivo* injectors in an animal model of chronic kidney disease, Apopt Syndrome).

## Results

Control: 2-D EVs, Volume: 40mL,  $3.07 \times 10^9$  EV/mL, Total EV:  $2 \times 10^{10}$

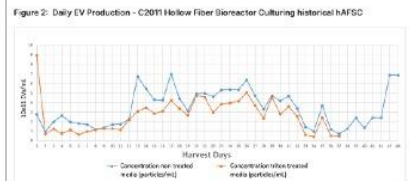
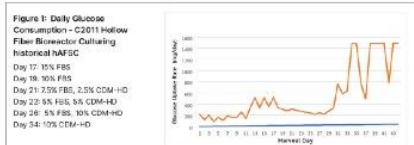


Figure 3 - hAFSC-EV size. Nanosight analysis EVs derived from 2-D (A) and 3-D (B) (all average size is 113 nm mode)

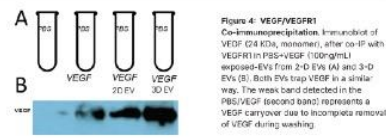
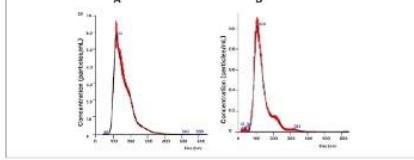


Figure 4: VEGF/VEGFR1 Co-immunoprecipitation. Immunoblot of VEGF (24 kDa, monomer), after co-IP with VEGFR1 in PBS+VEGF (100ng/mL) exposed-EVs from 2-D EVs (A) and 3-D EVs (B). Both EVs trap VEGF in a similar way. The weak band detected in the PBS/VEGF (second lane) represents a VEGF carrier due to incomplete removal of VEGF during washing.

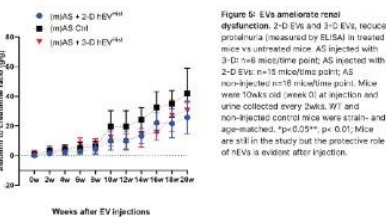
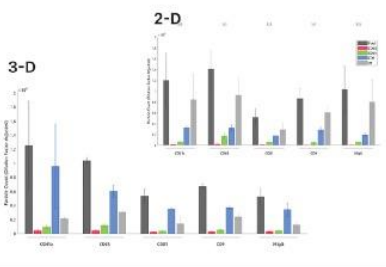


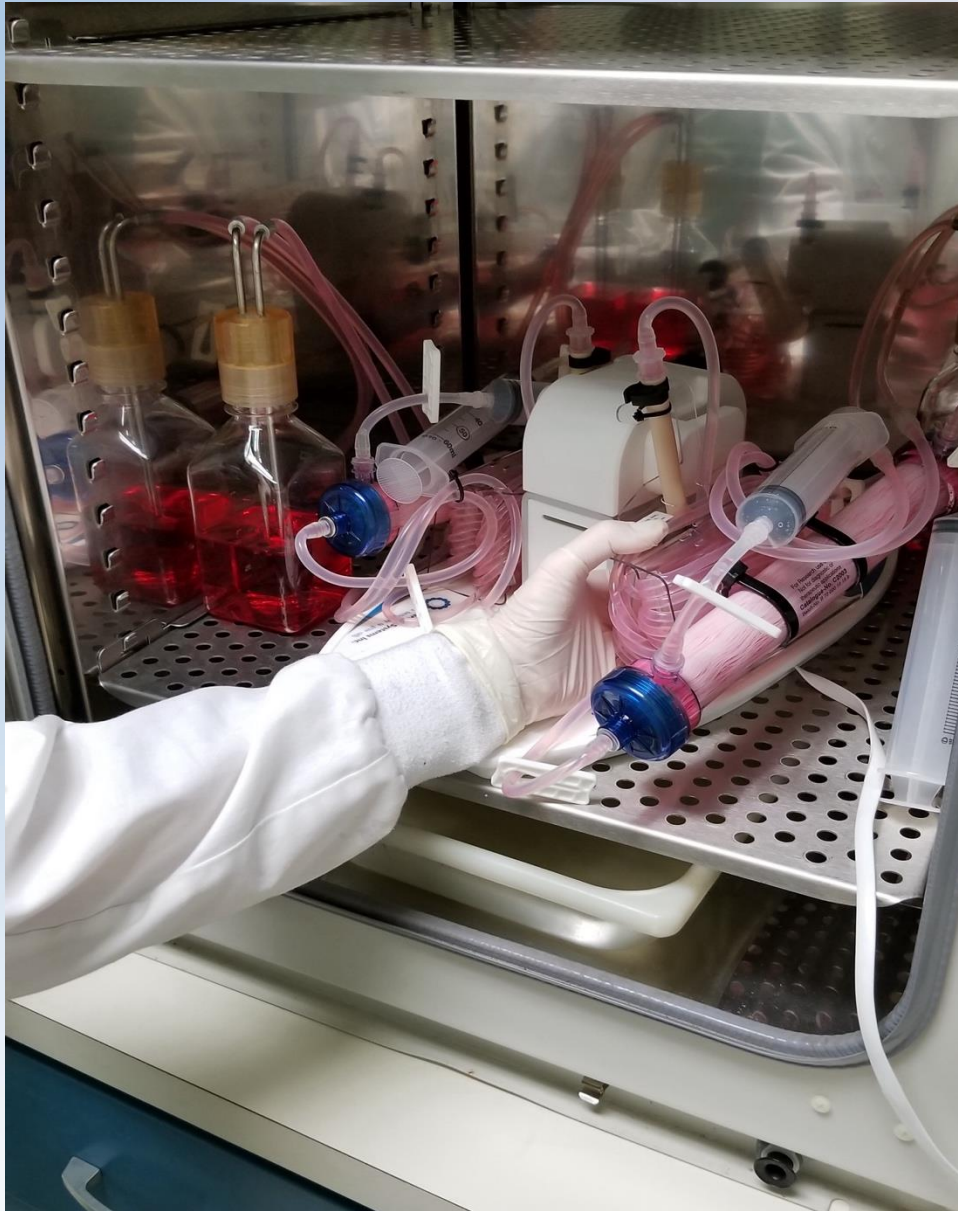
Figure 6: 3-D hEVs present similar tetraspanin profile of 2-D EVs as evaluated by ExoView



## Discussion and Conclusion

3-D EVs had comparable properties and bio-activity relative to 2-D EVs, but the HFBR produced 100x more concentrated EVs per mL. Each daily harvest produced more than  $10^{10}$  EVs,  $10^{10}$  would be an estimated human dose. The adaptation of these cells to a chemically defined medium and the demonstrated production range represents a significant step towards enabling therapeutic applications of hAFSC for treating kidney diseases in humans. The C2018 hollow fiber bioreactor module represents an additional six scale-up from the data presented here. The HFBR is a closed system that can be cGMP compliant. In conclusion, the HFBR can produce sufficient numbers of EV to support pre-clinical and clinical applications of EVs with at least similar properties to EVs produced by conventional 2-D methods.

# Scale-up Options in 3-D Cell Culture



**From  
Impossible to  
3,000 Doses a  
Month.**

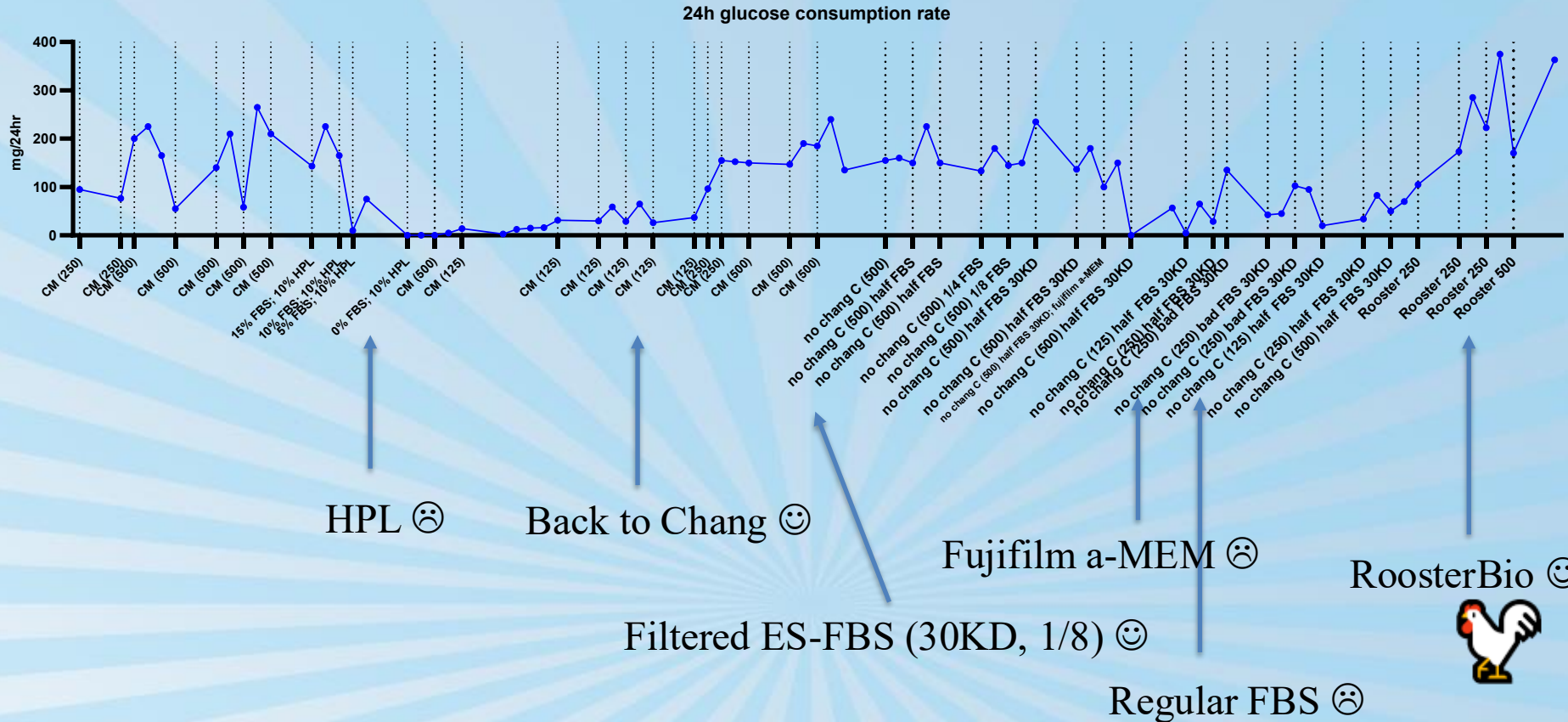


# What is it? See the Unseen!

One week EV harvest of  
EVs from DiFi cells.  
Can produce GRAM  
quantities of EVs



# 110 Days Continuous Production of EVs from HAFSC

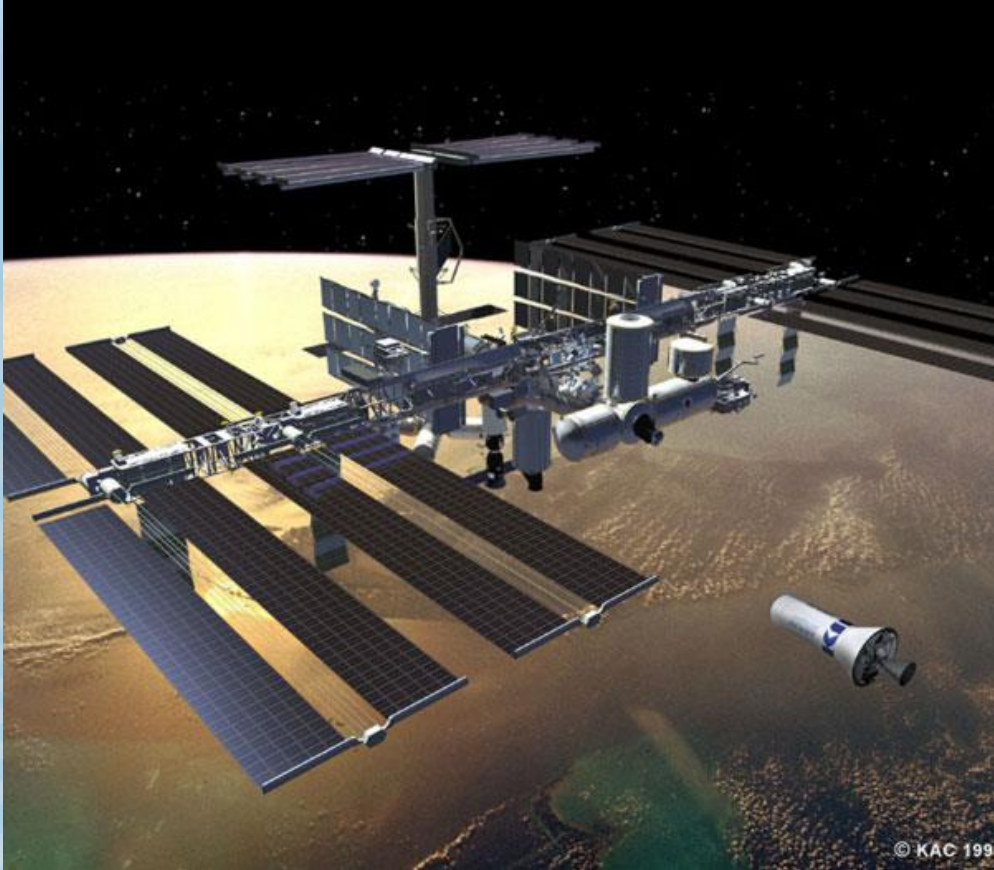
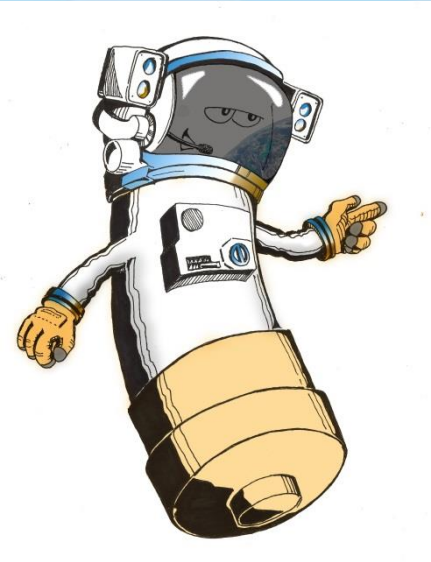


Data courtesy of Dr. Laura Perin and Paolo Neviani,  
Children's Hospital, Los Angeles.

# Summary

- Hollow fiber bioreactors are the method of choice for the culture of  $10^9$  to  $10^{11}$  cells
- Can produce gram quantities of exosomes
- Concentration of 100x higher than with conventional methods
- The most *in vivo* method for culturing cells over long periods of time
- Suitable for cGMP production
- Permits use of FBS without endogenous EV contamination
- Enhanced bioactivity
- Saves time, space, purification costs

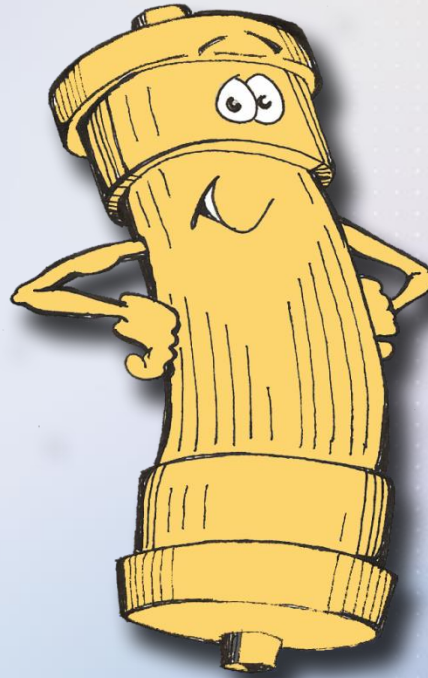
# FiberCell Systems HFBR in Space



“...The evolution of cell culture”

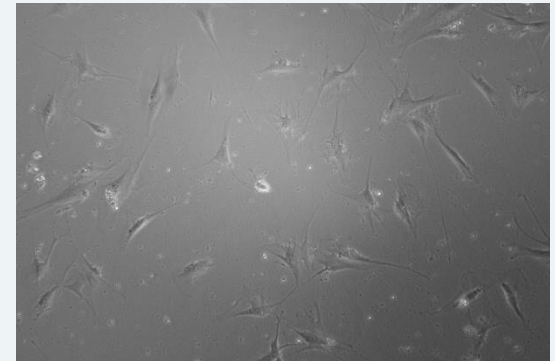
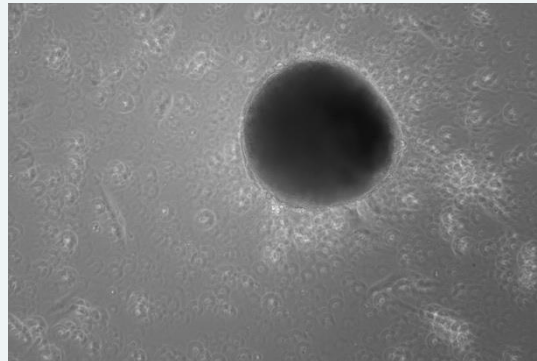
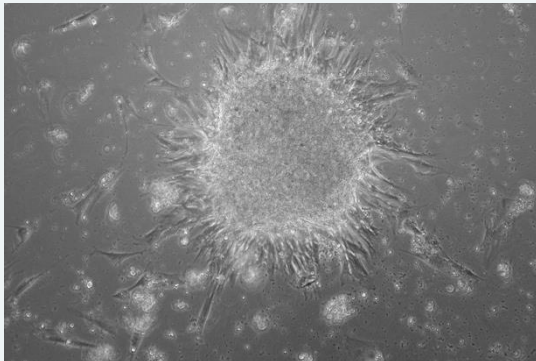
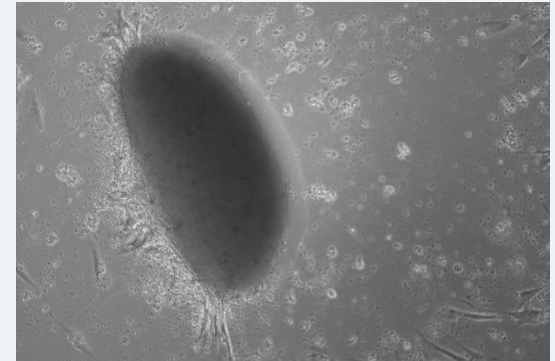
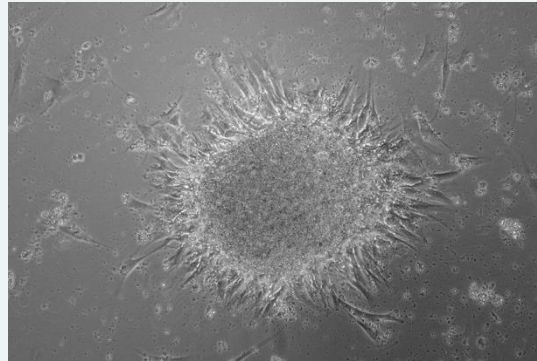
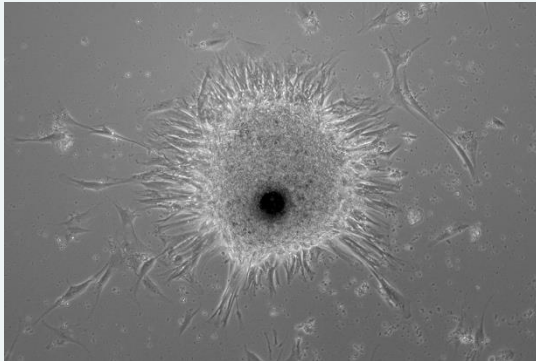


Thank you.



# Cell Harvest After 65 Days of Culture

TrypLE 20 min, spin, plate

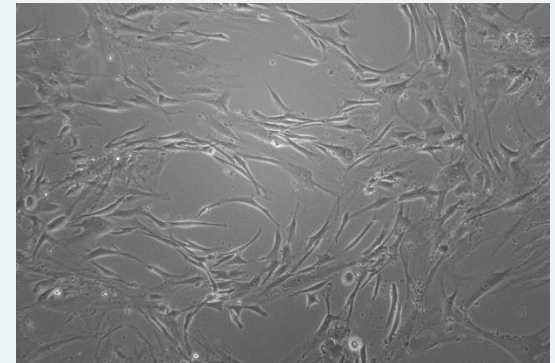
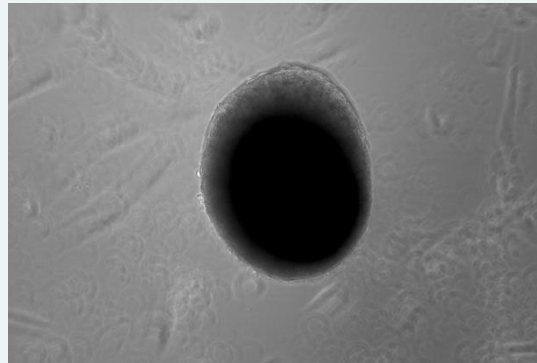
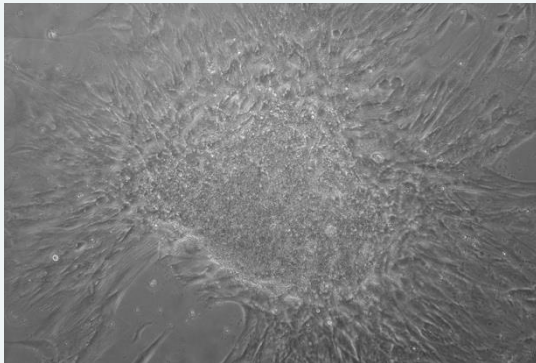
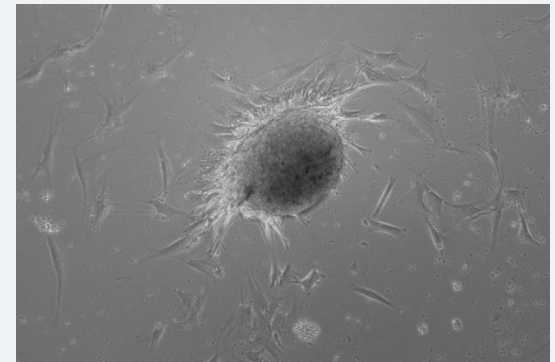
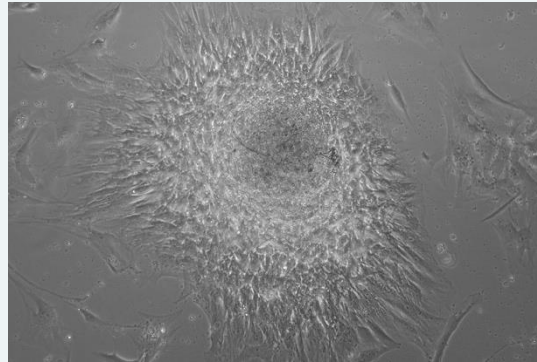
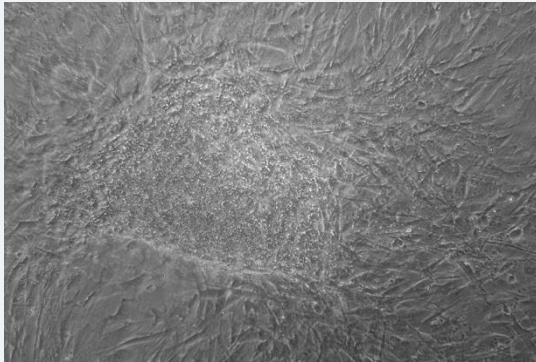


**Conclusion:**

There are indeed spheroids in the FiberCell bioreactor

# Cell Harvest After 2 Days of Culture

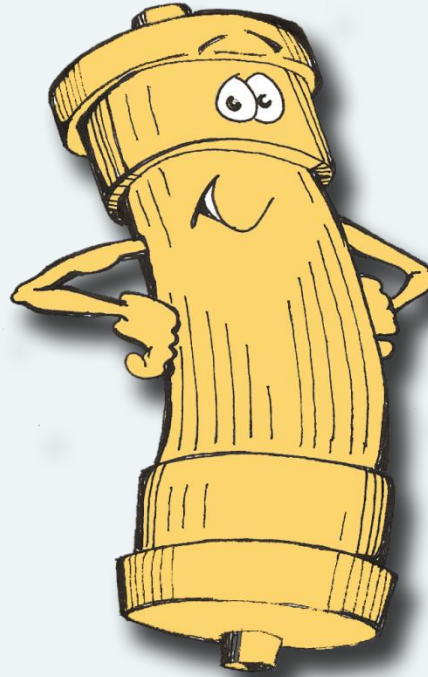
TrypLE 20 min, spin, plate



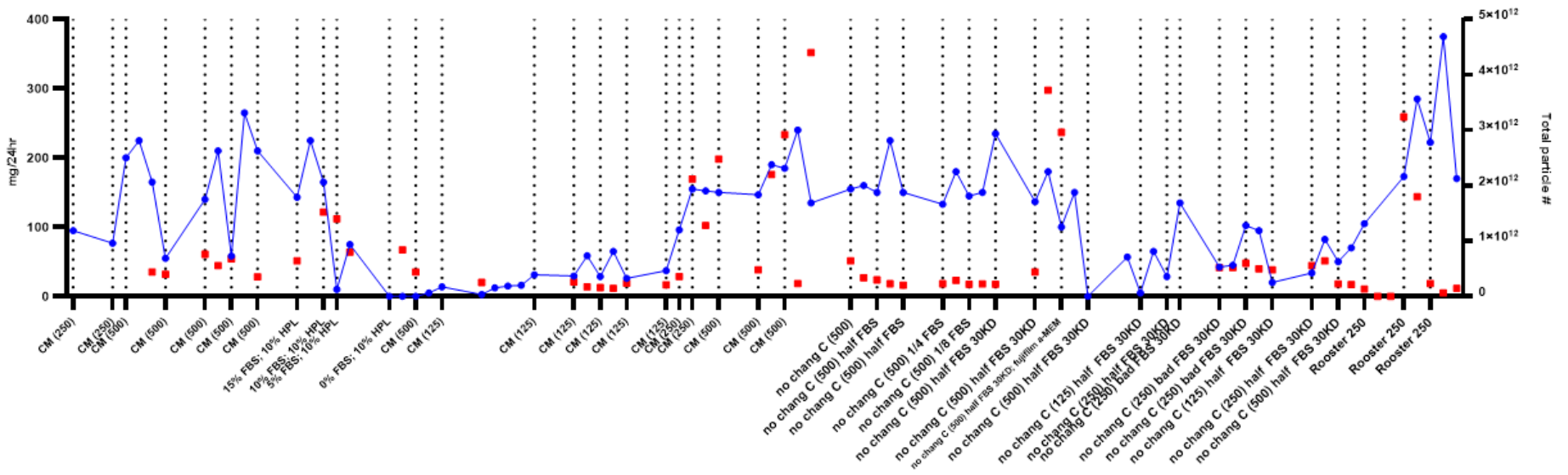
**Conclusion:**

Spheroids spread and cells migrate and proliferate

Thank you.



—●— Glucose consumption  
 ■ EV yield by SEC



# 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
- MSC proliferation may be limited
- Easy to apply defined stressor such as temperature or anoxia



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A better way to grow cells.  
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GROW CELL  
Certified Nutrient  
**1X PEB**  
(Phosphate Buffered)  
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Volume:  
Lot No.:  
Expires:  
Store:  
pH: