

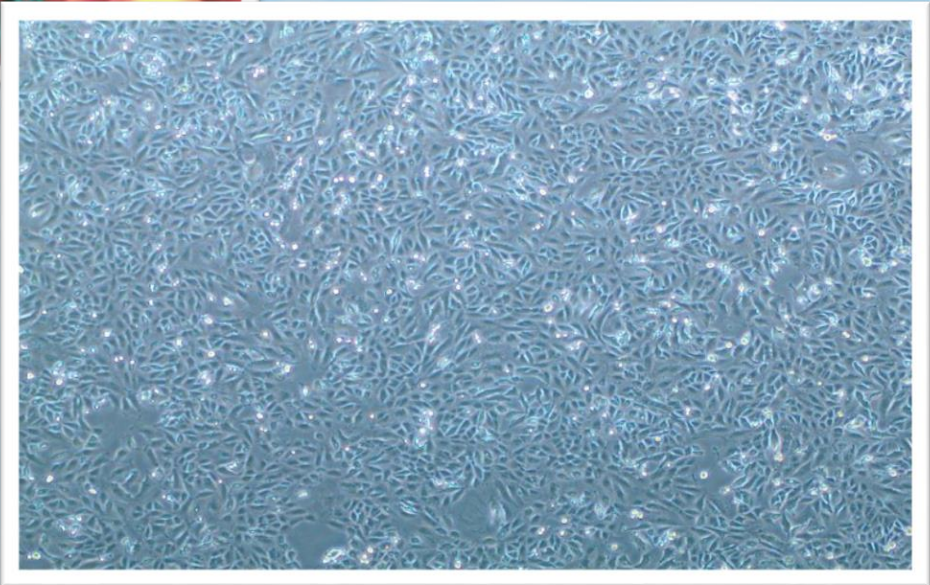
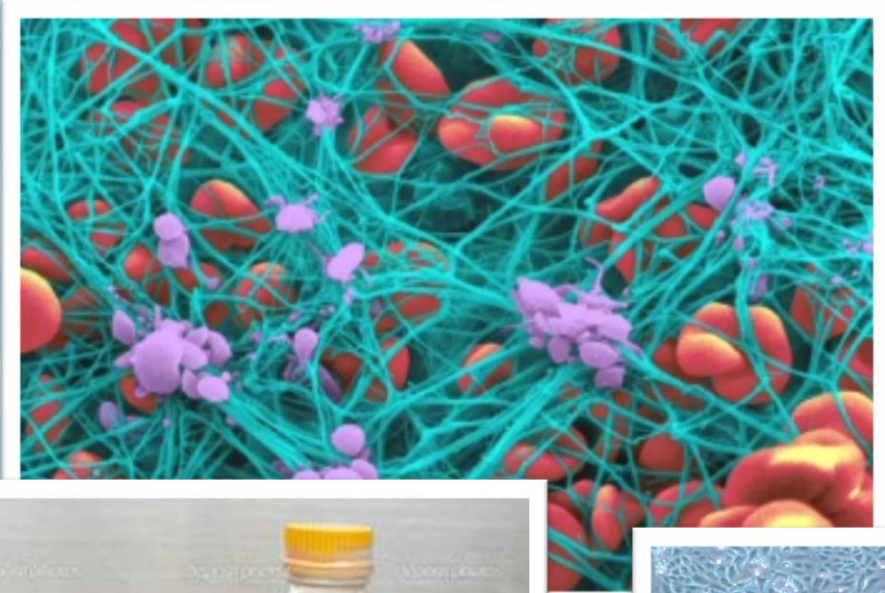


Production of Exosomes and Culture of Stem Cells in Hollow Fiber Bioreactors

By John J.S. Cadwell



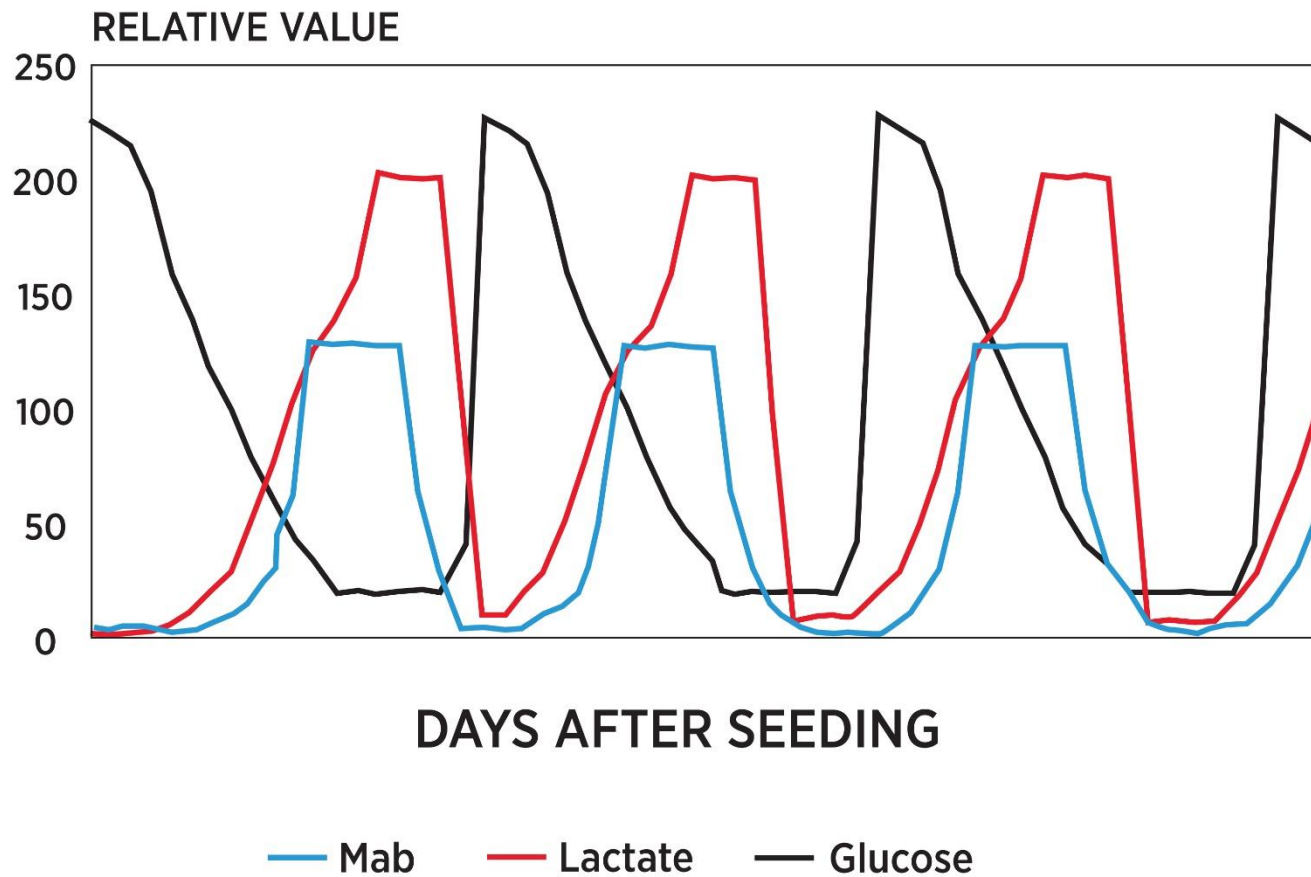
Cell Culture Through the Ages



Cell Culture Options for Scale-Up

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

“Feast or Famine”



Current Method

- 1) Expand cells in flasks
- 2) Remove serum
- 3) Collect exosomes for 2 days
- 4) Rinse and repeat



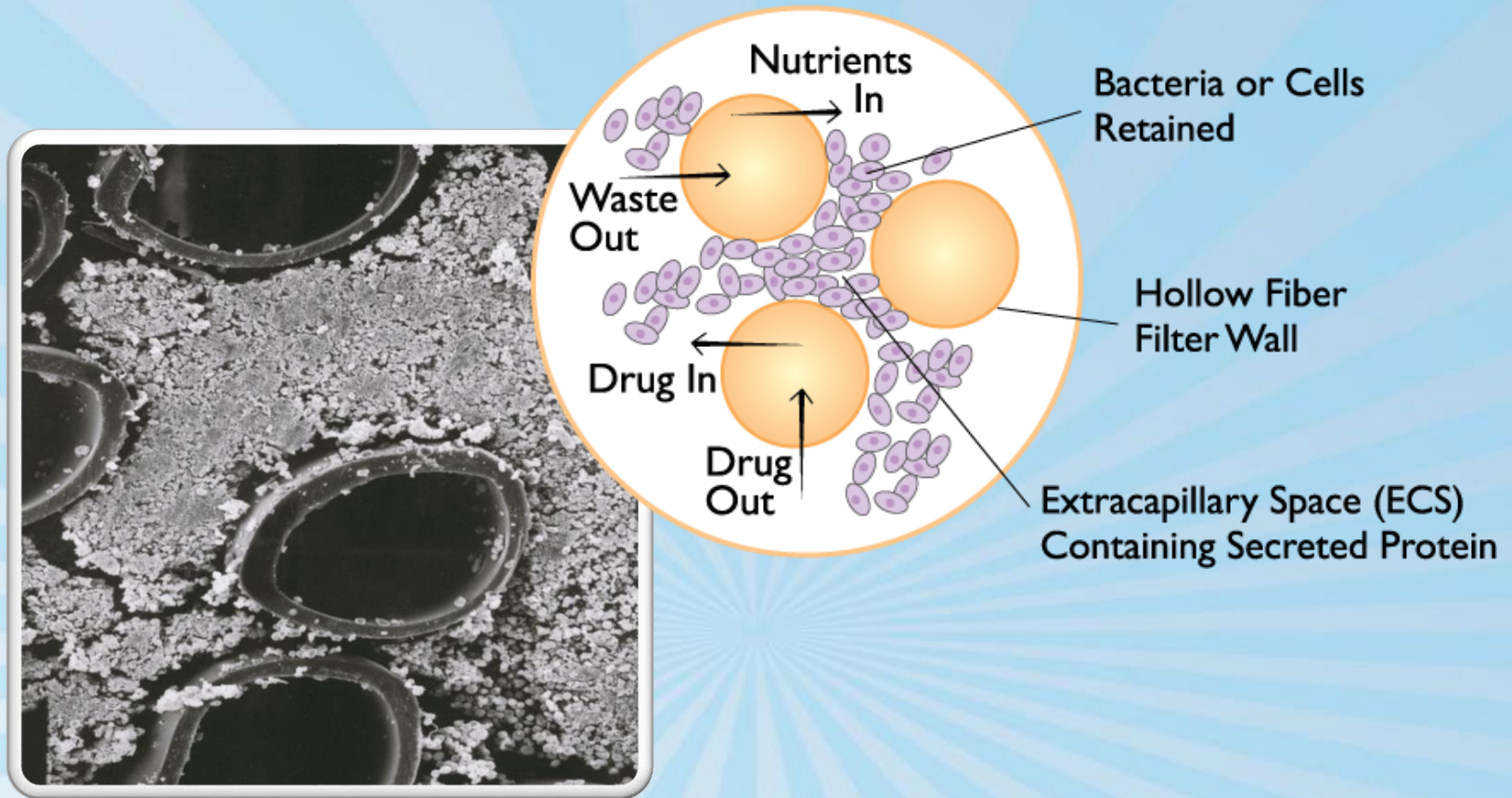
Requirements for standards

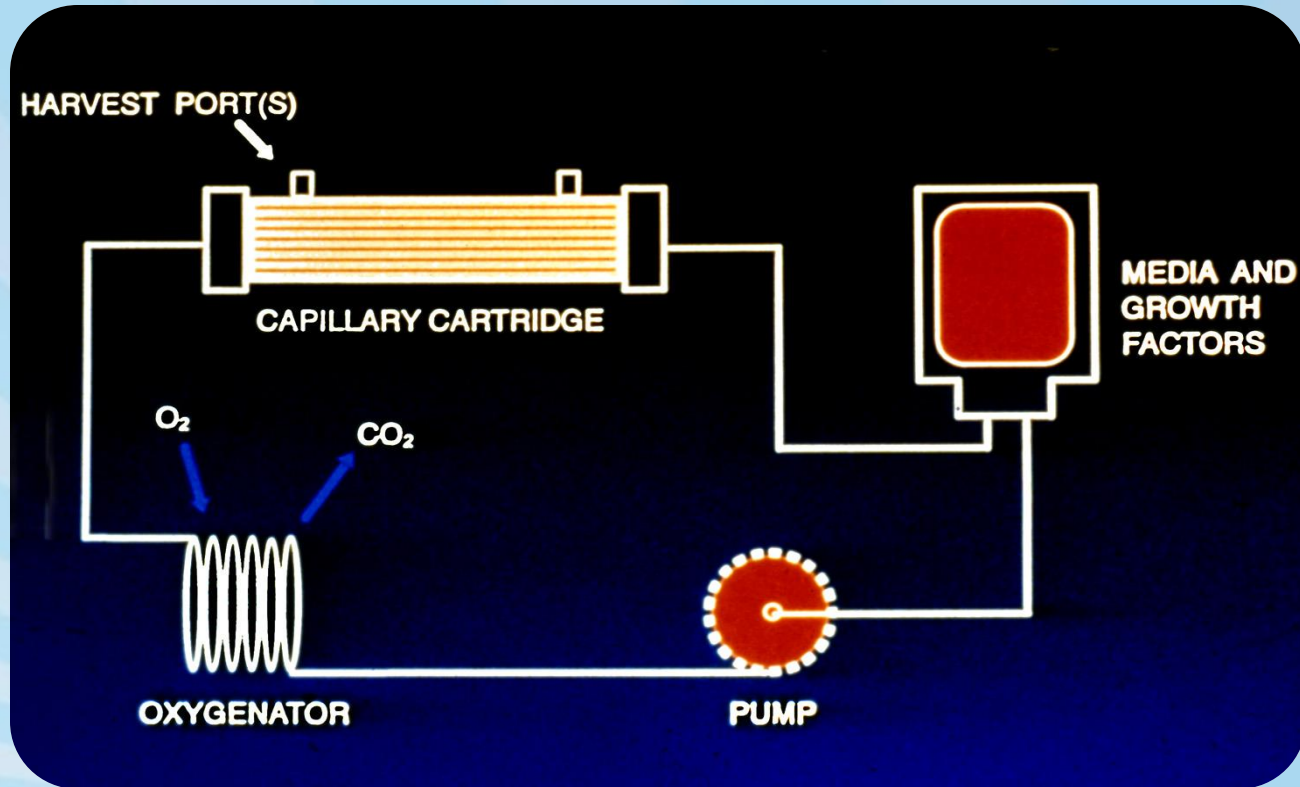
- 1) Consistent and uniform
- 2) Comparable to *in vivo*
- 3) At scale
- 4) Concentrated
- 5) Contaminant free

Sources

- Primary cells
- Engineered cell lines for recombinant EV production.

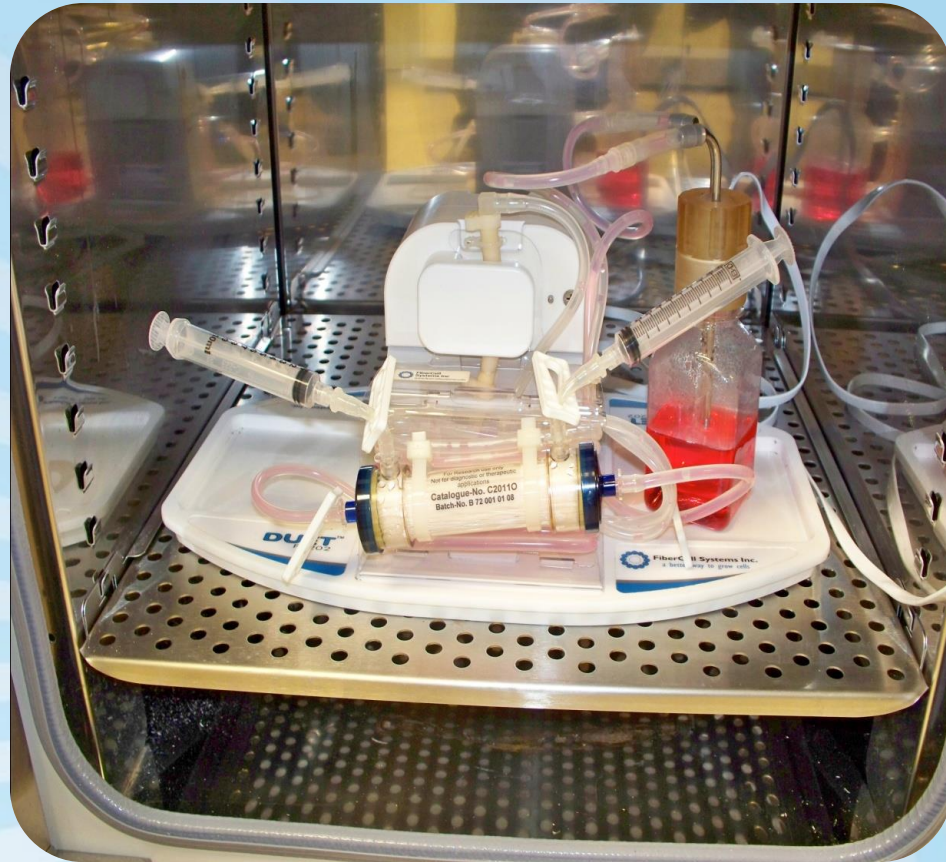
Hollow Fiber: How it works





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

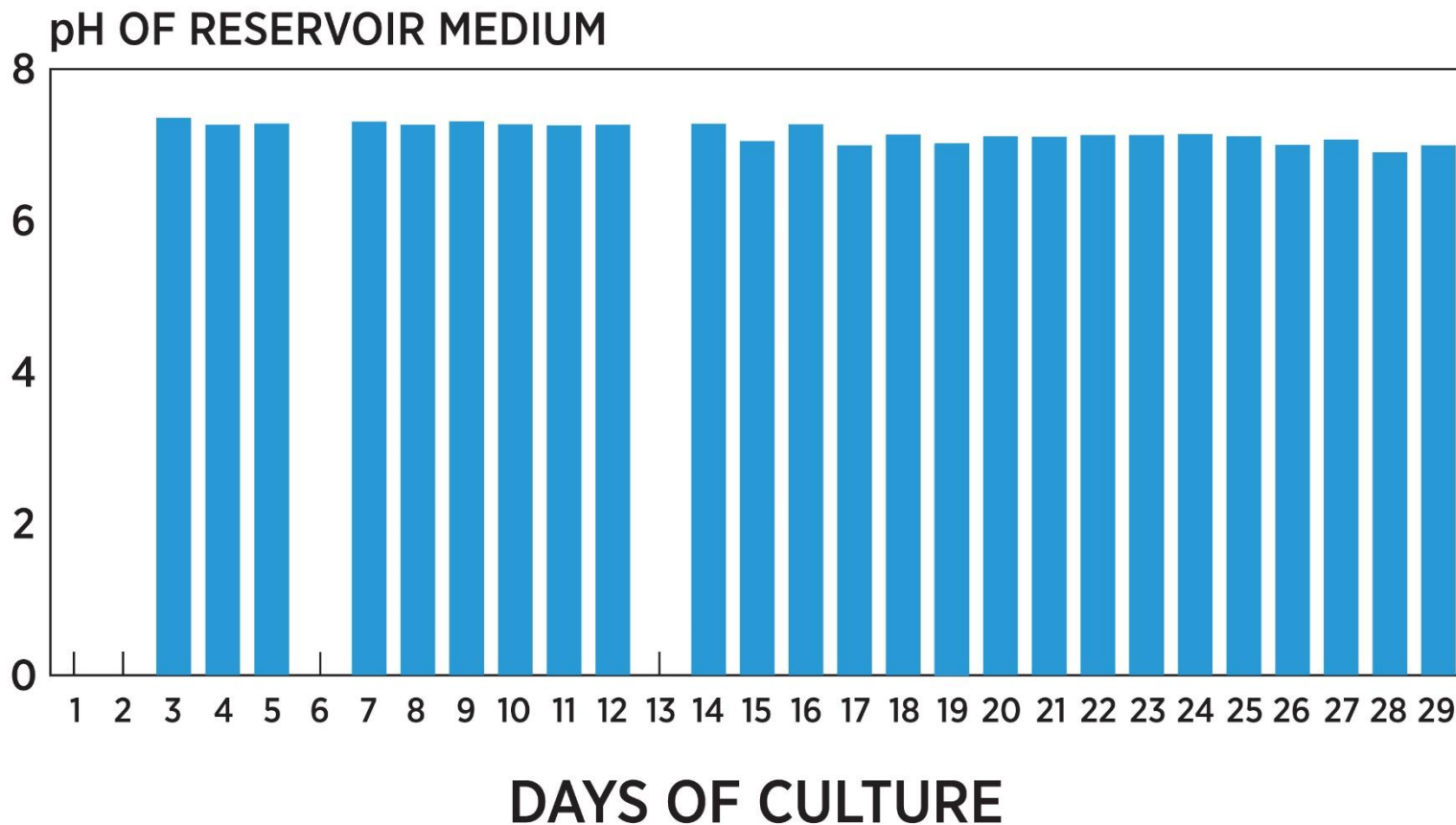
In the Laboratory



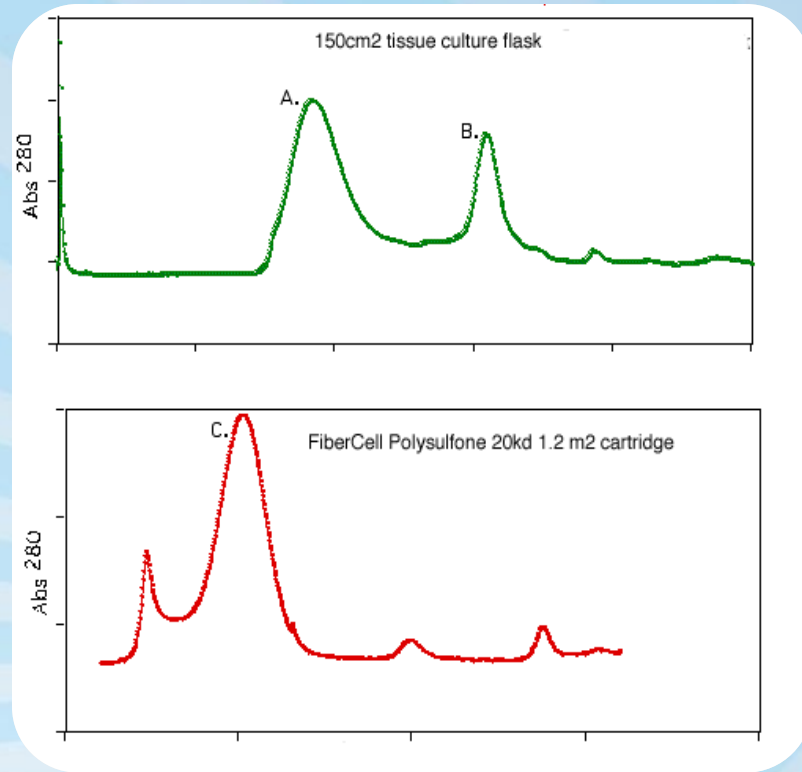
- Fits in any standard sized incubator
- Gas controlled by incubator
- Temperature controlled by incubator
- Thin cord for power

HF CULTURE OF CHO CELLS

pH CHANGES

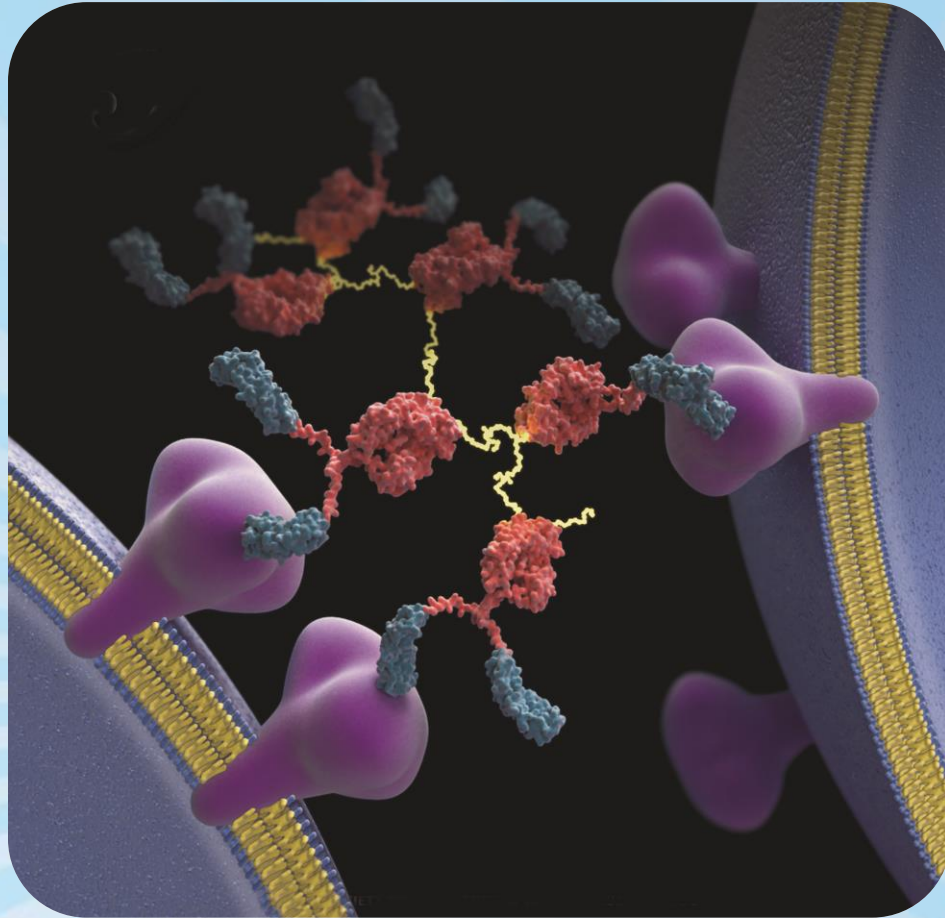


Recombinant Protein Production



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

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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

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

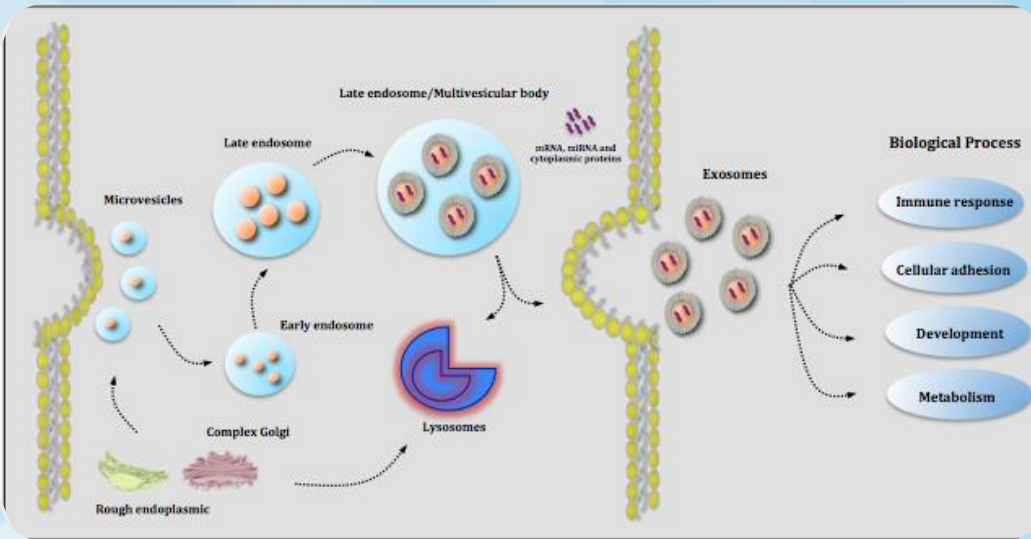
Advantages of Hollow Fiber Cell Culture

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

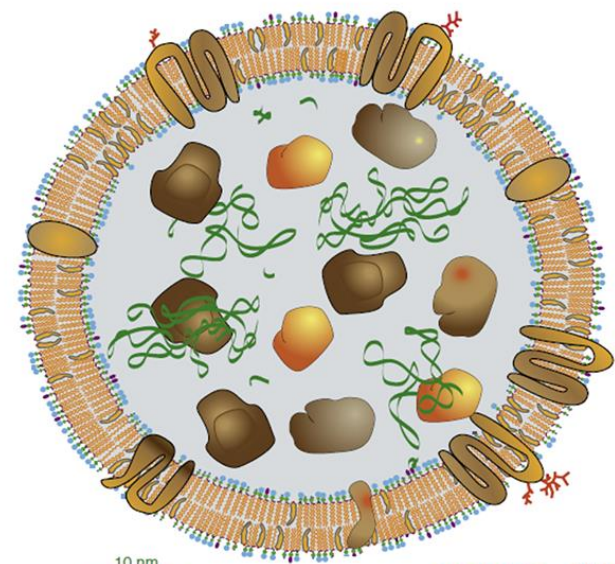


Exosomes

- 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



The Anatomy of an Exosome



- Ceramide
- Sphingomyelin
- Phosphatidylserine
- Phosphatidylethanolamine
- lyso-Phosphatidylcholine
- Phosphatidylcholine
- Phosphatidylinositol
- Cholesterol

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

Human Adipose Derived Adult MSC

130 T225 Flasks

- Harvest Volume: 4000mls
- Protein: 0.9 mgs
- Particles: 1.6×10^9

One C2011 Bioreactor

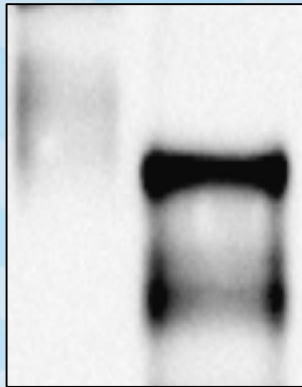
- Harvest Volume: 120mls
- Protein: 14.45 mgs
- Particles: 3.27×10^{12}

5×10^8 cells seeded

Bioreactor is a rich source of exosomes

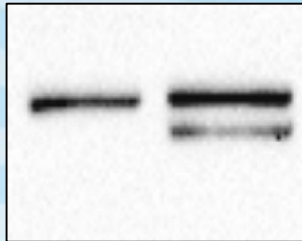
Flask
exosomes

Bioreactor
exosomes



CD63

7.6-fold
increase



Alix

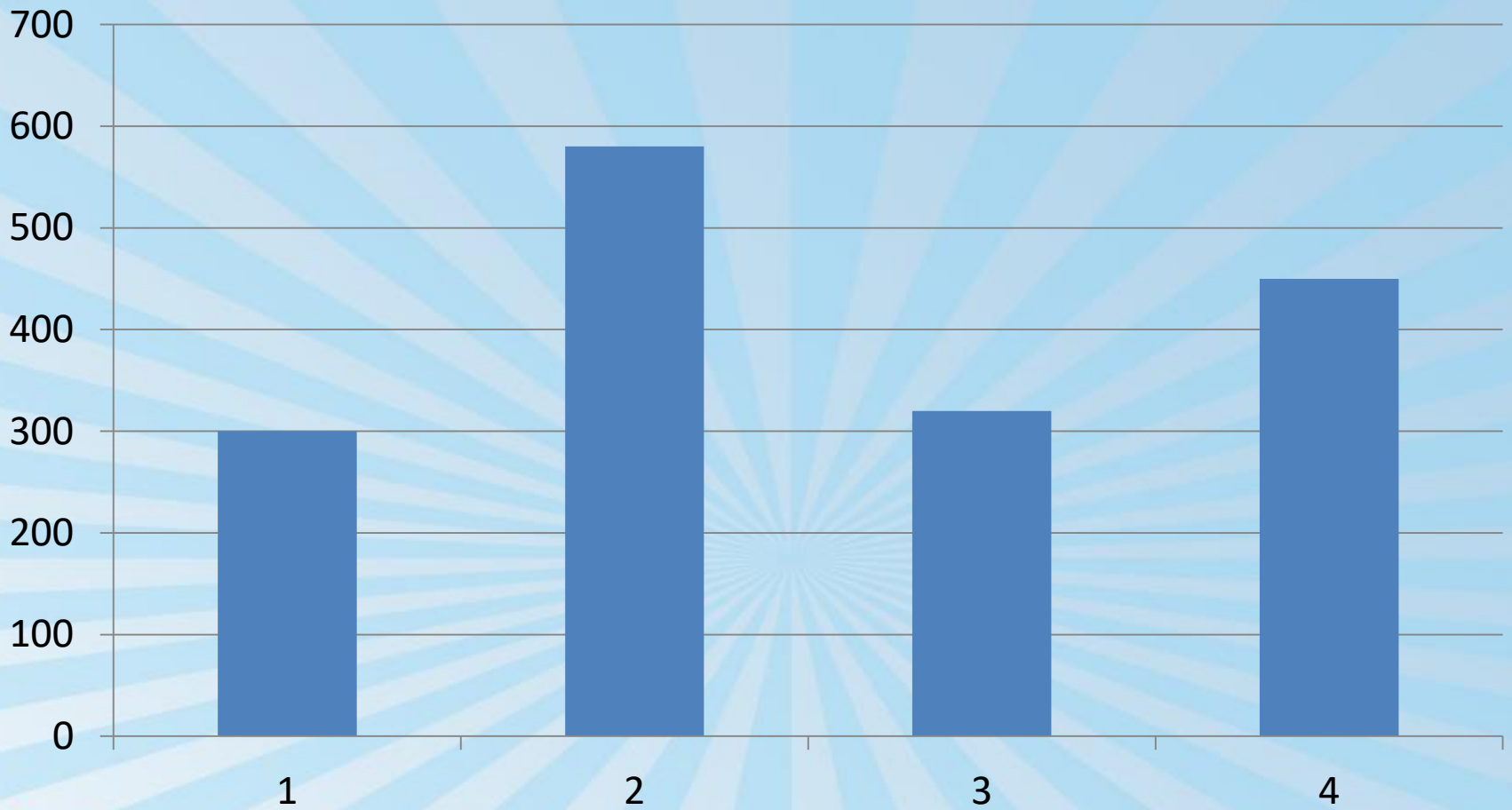
2.1-fold
increase

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**

Culture Platform (1 x 10⁹ cells)	Medium Volume (mL)	Total Number of EVs (10⁹)
Bioreactor Harvest #1	40	320
Bioreactor Harvest #2	40	250
Bioreactor Harvest #3	40	290
130 Flasks (225 cm ²)	4000	16



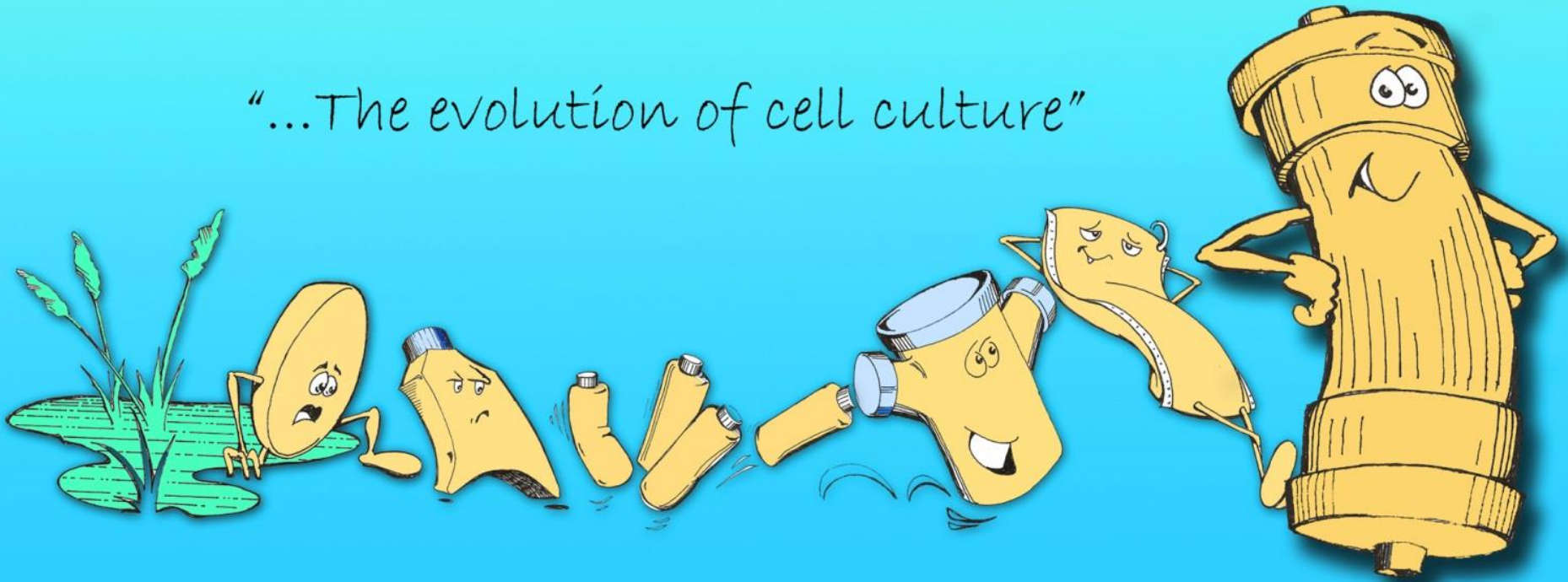




Summary

- 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
- Saves time, space, purification costs
- A practical method for generating EV standards and potential therapeutics

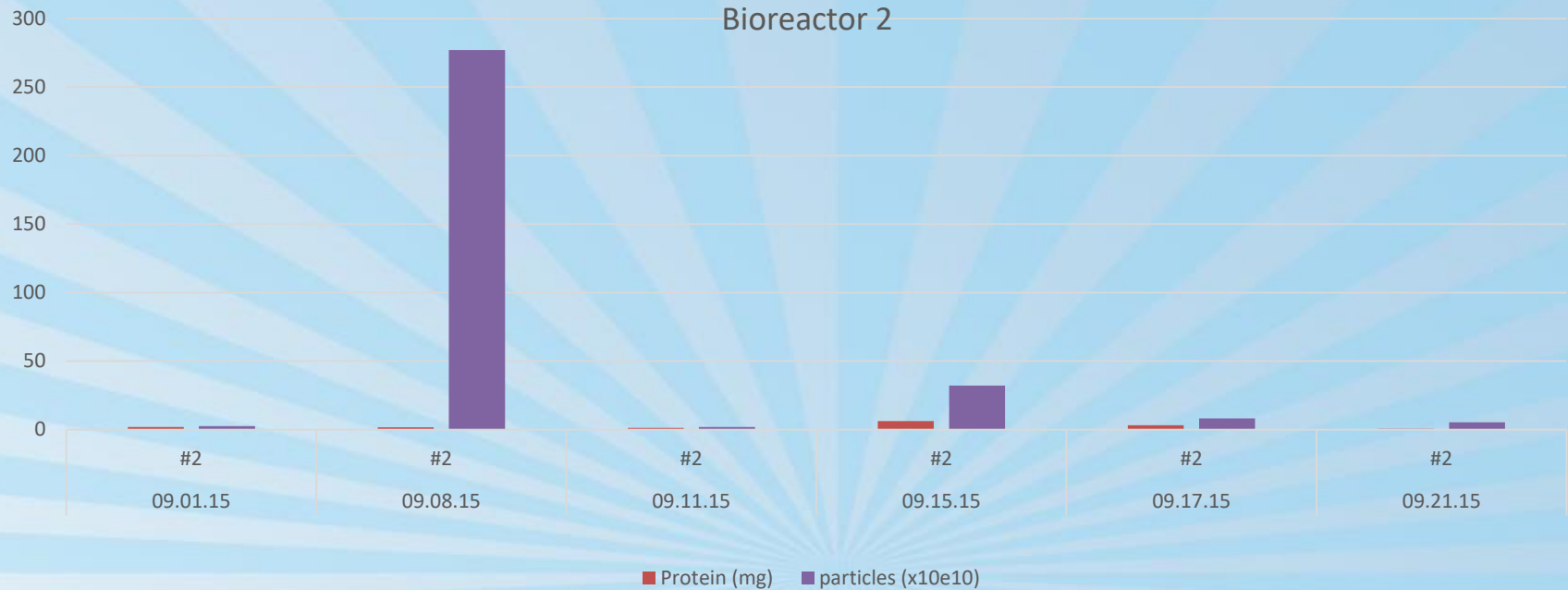
“...The evolution of cell culture”



Thank you.



Exosomes from Adult Adipose MSC



Total Volume: 120 mls

Total Protein: 14.45 mgs

Total Particles: 3.26 X 10e12

1.5 mgs/liter