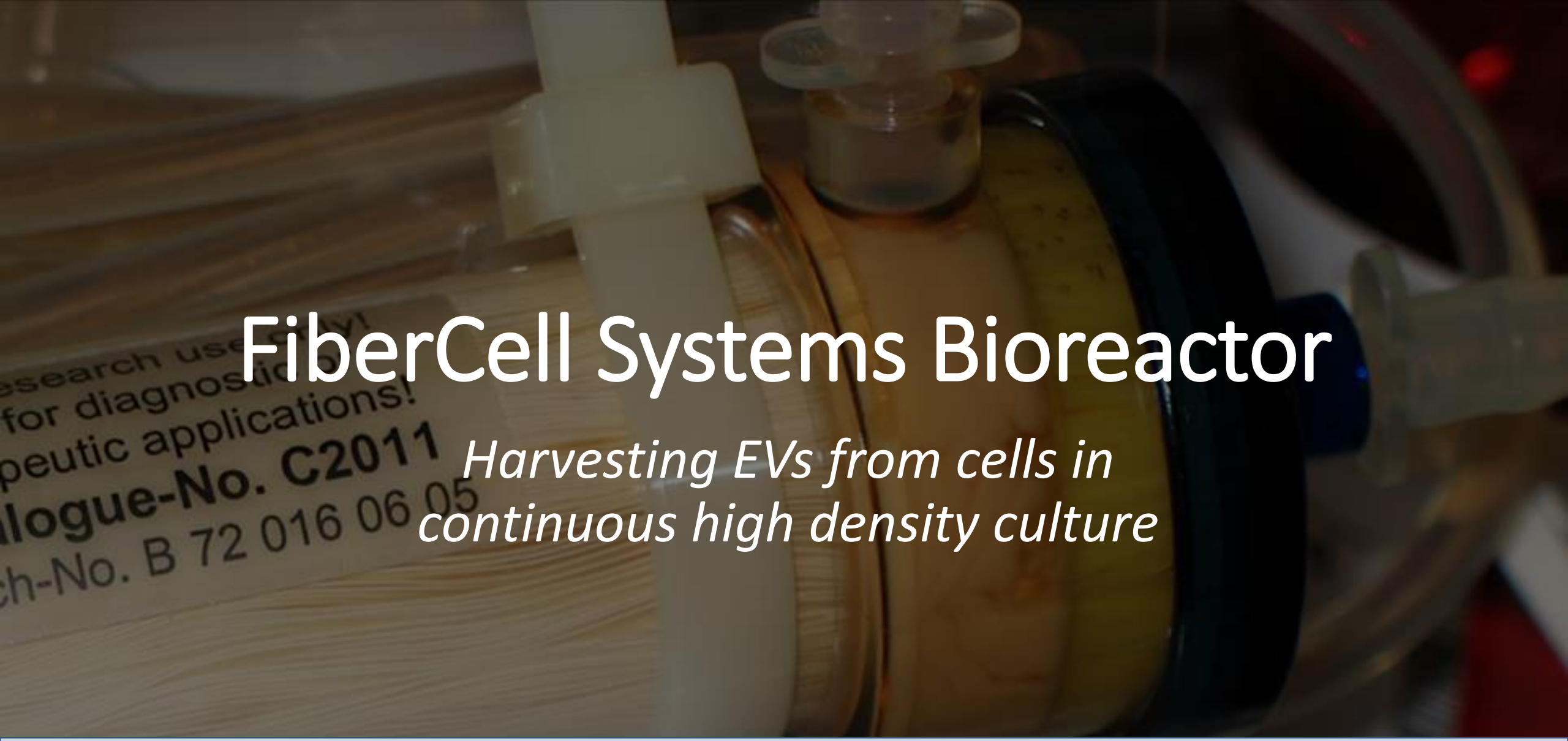

FiberCell Systems Bioreactor

Harvesting EVs from cells in continuous high density culture

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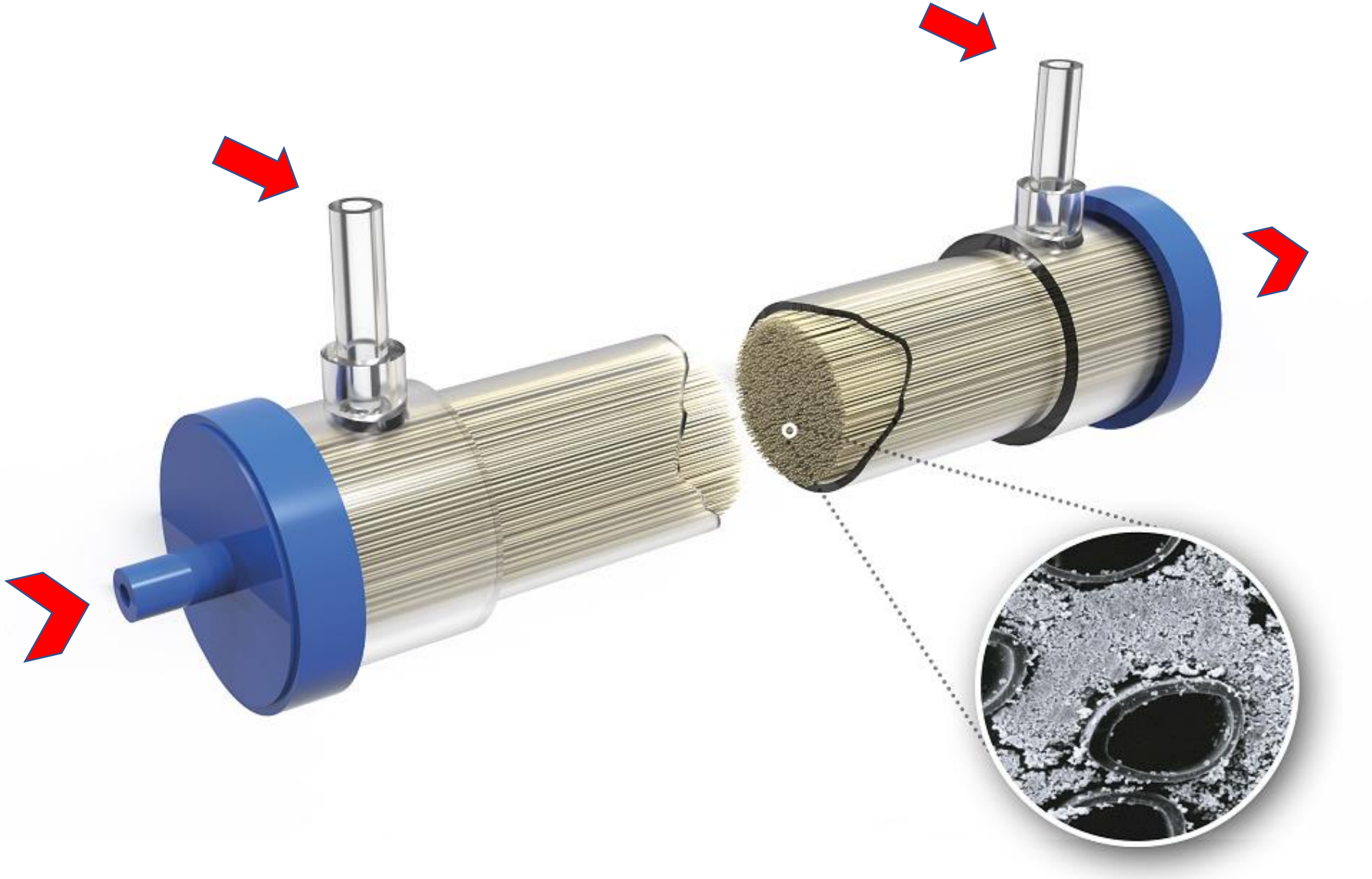
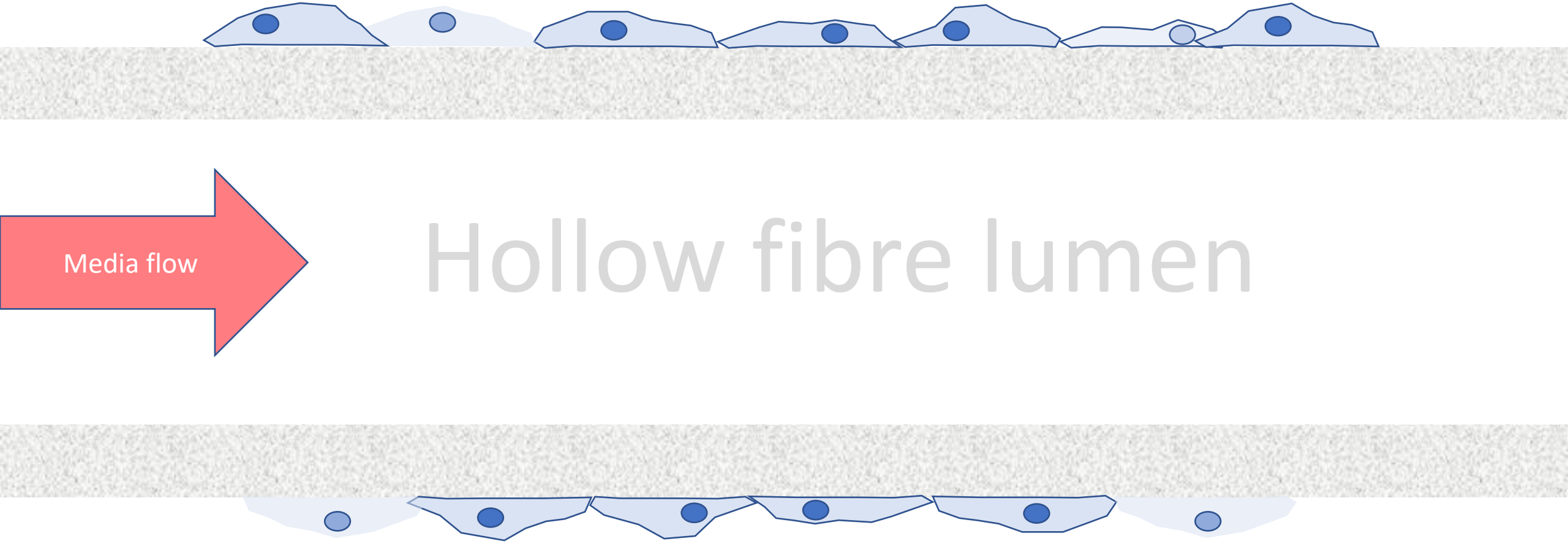
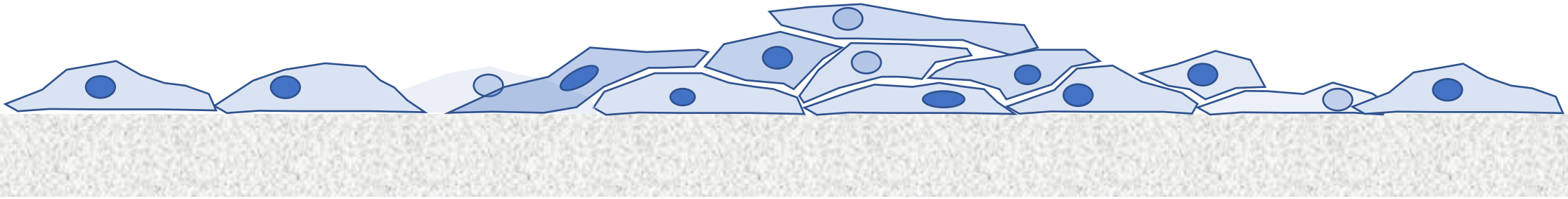


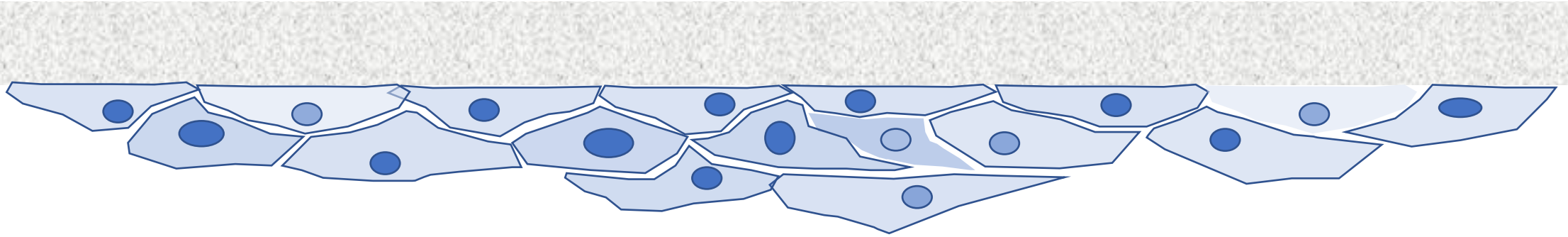
Cells grow on a **POROUS** substrate



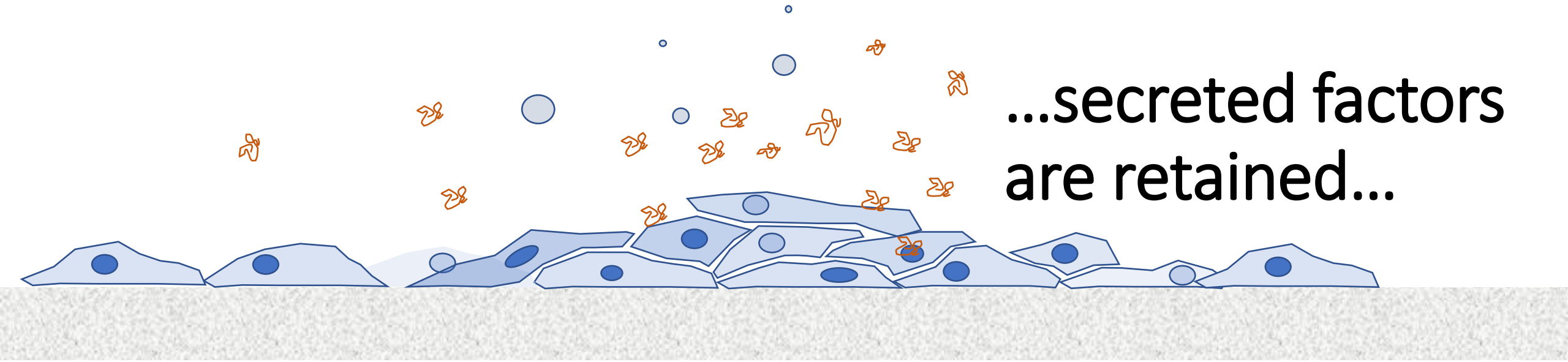
Cells can grow post-confluently in 3D layers



Hollow fibre lumen



5 kDa or **20 kDa** molecular weight cutoff

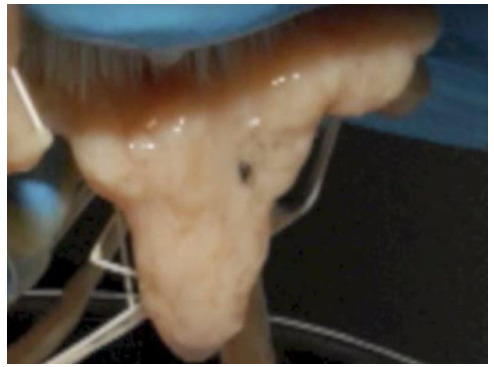


…secreted factors are retained…

Media flow

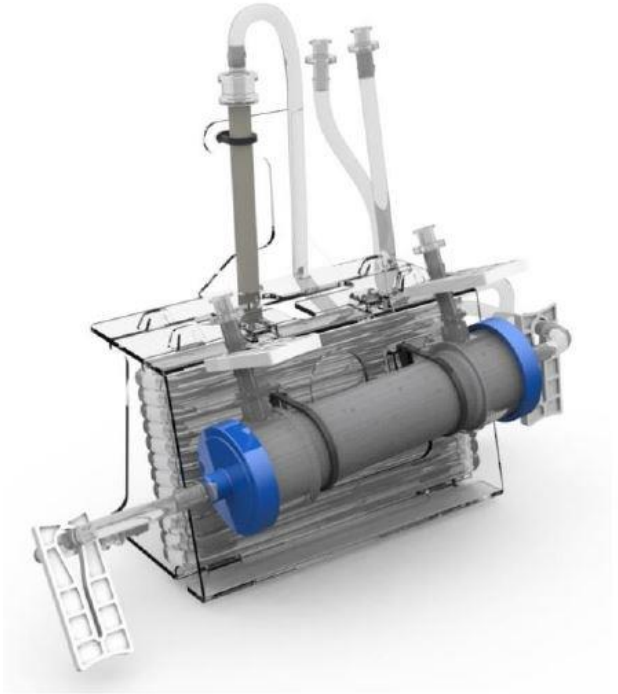
…but some smaller proteins might diffuse away

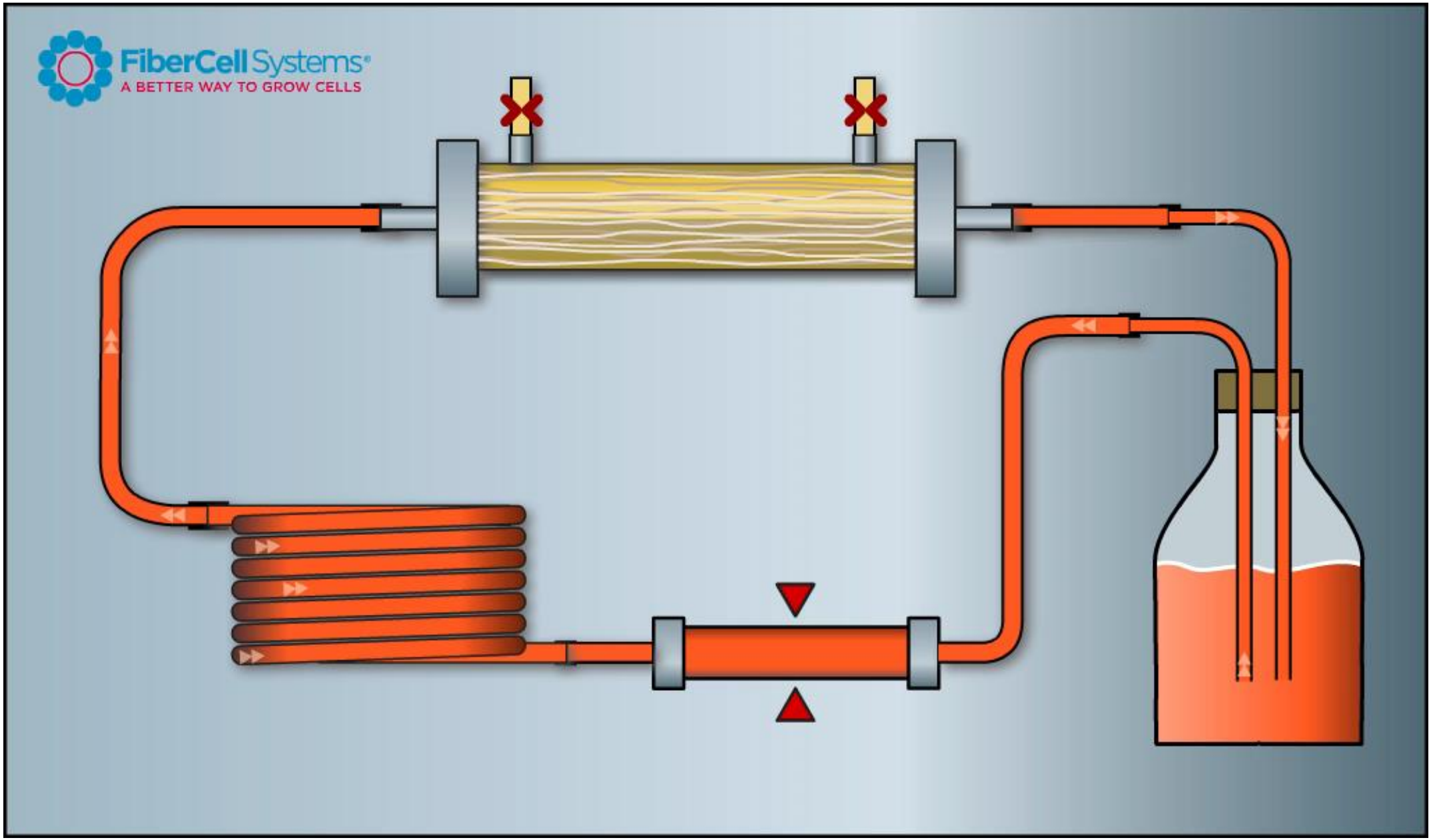
Tissue-like density at end-caps and between fibres



LNCap prostate cancer line in hollow fibre

Compact single-use cartridge unit

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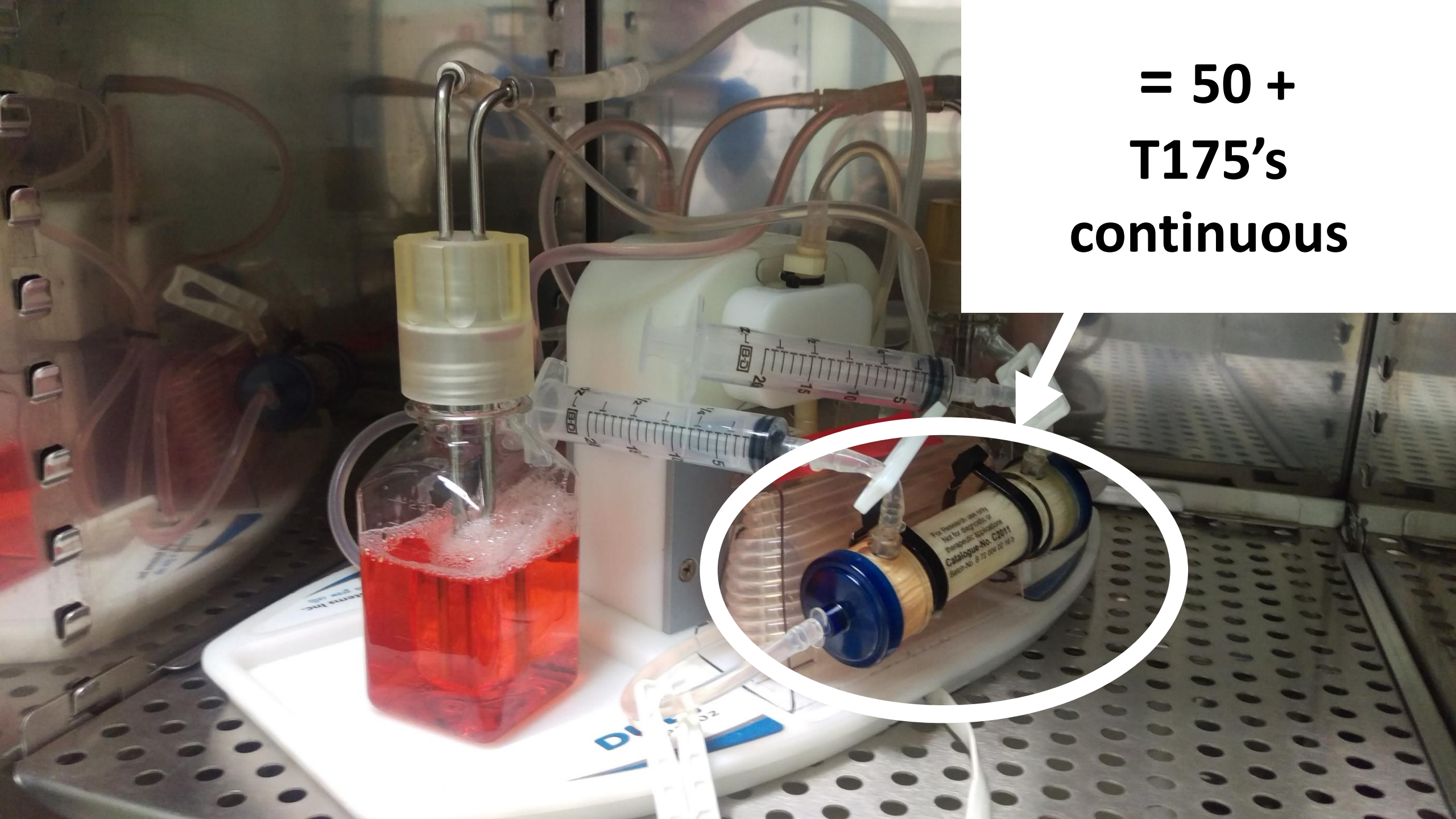
Duet pump


= 50 +
T175's
continuous

High yield supernatant

Every day

No passaging

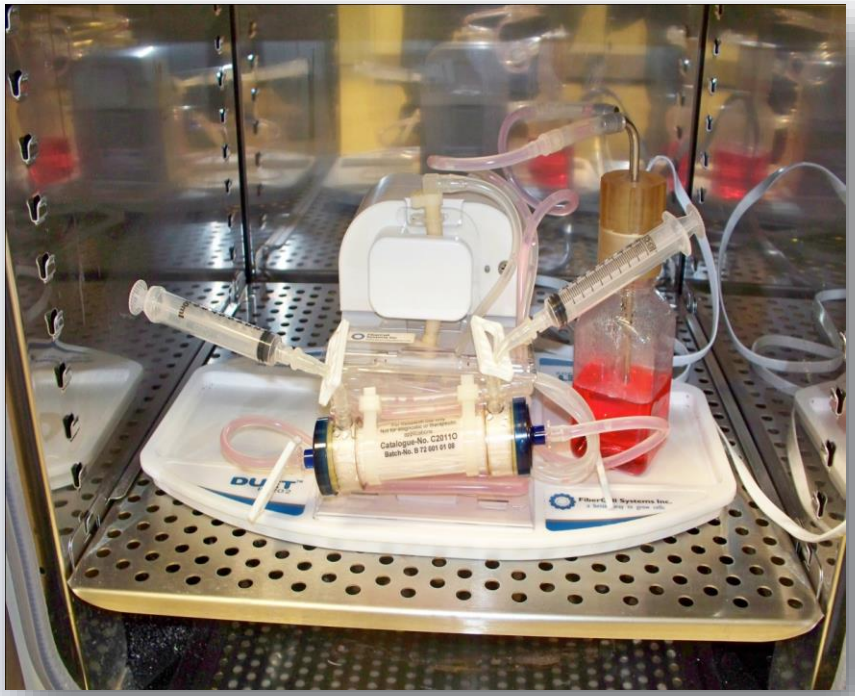
Reduced trash

In the Laboratory…

Fits on one shelf of 180L incubator

Incubator controls gas and temperature

Power cord very thin



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Practical aspects

Work with cartridge/reservoir in laminar flow cabinet

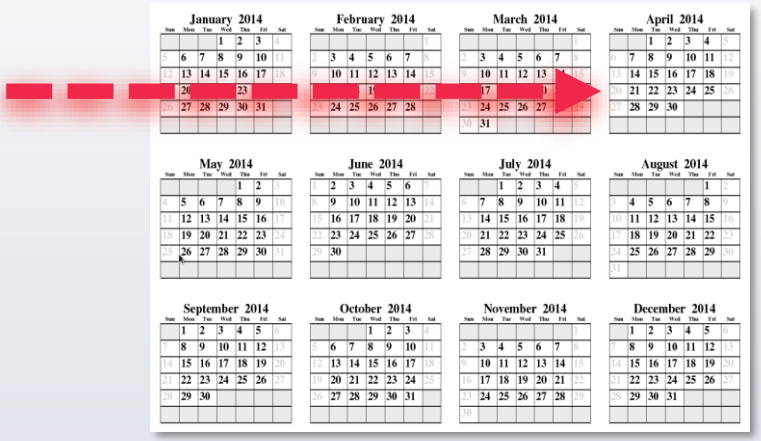
Harvest product using syringes

Measure glucose consumption

15 minutes per day

General Advantages of hollow fibre bioreactors

- Compact – 100 X cell density vs. flasks (...and no passaging)
- Concentrated product
- Large amounts of product (time scaleable process)
- Easier to switch cells to chemically defined media
- No shear stress
- More *in vivo*-like conditions for better quality of cell-secreted products



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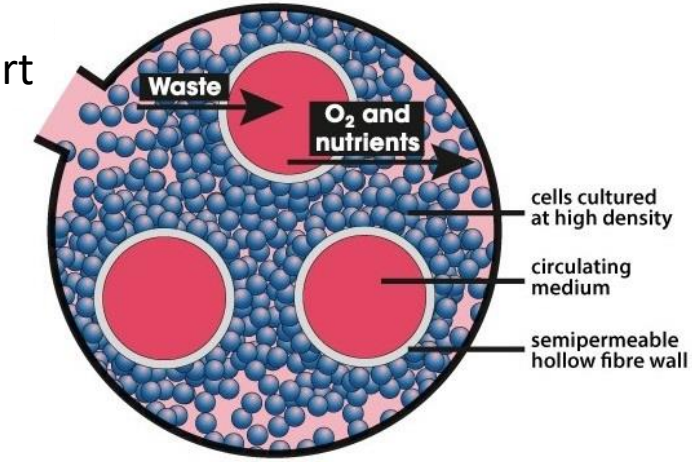
stress treatments

3D hollow fiber cell culture

Normoxia 10^9 cells → Hypoxia

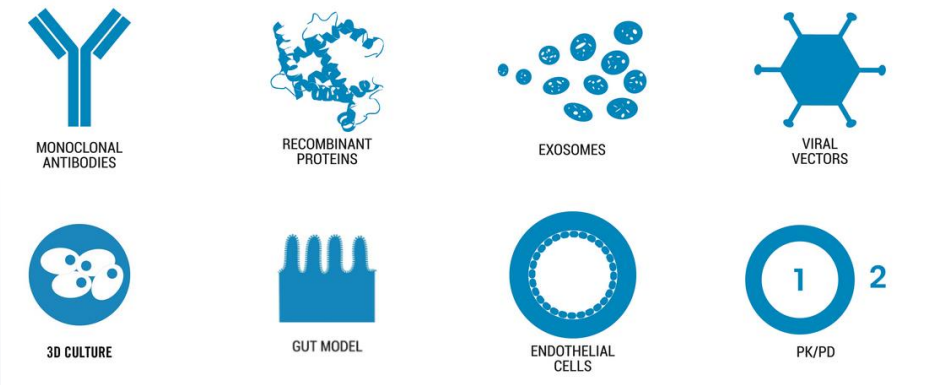
EV harvesting port



Alternative stressors :

starvation, temperature shift, chemical treatment, pressure pulsing

Hollow Fiber Applications



- Monoclonal antibody production
- Recombinant protein production
- Cell secretome – 3D *in vitro* models
- EV / Exosome production
- Endothelial cell culture under shear stress
- Cell co-cultivation
- in vitro toxicology

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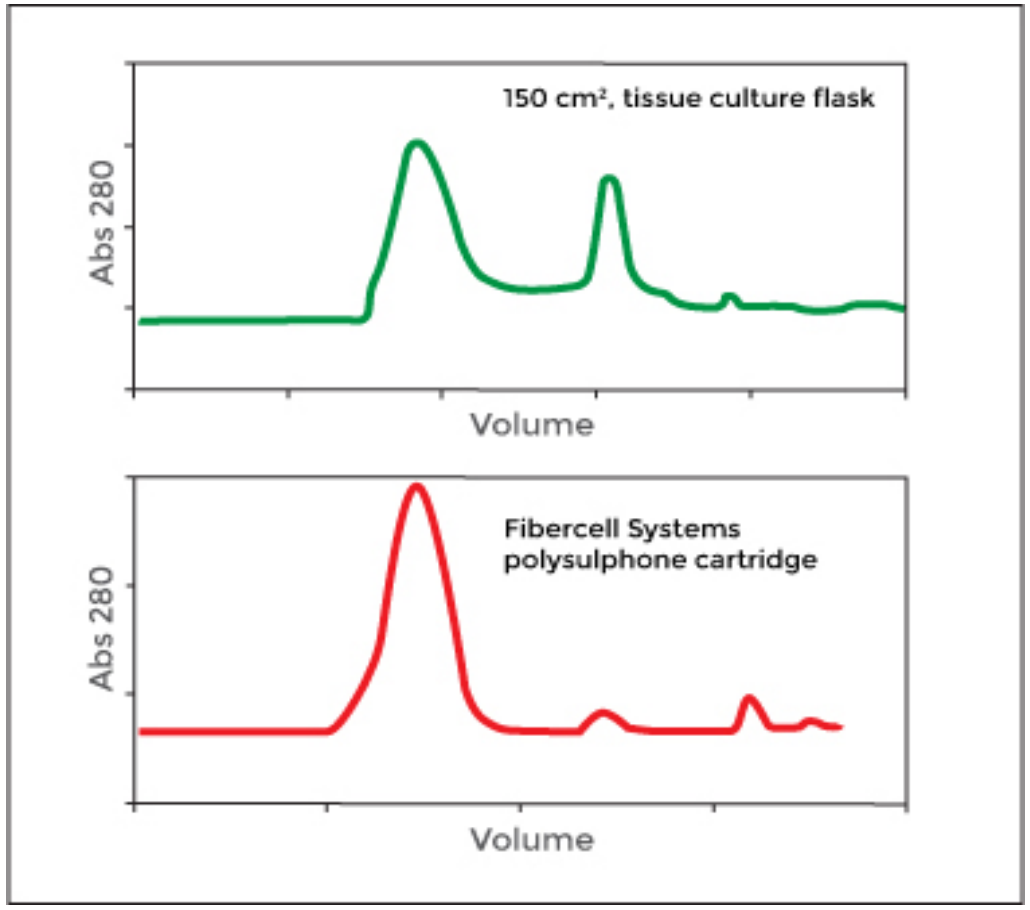
Biochemical and biological characterization of a dodecameric CD4-Ig fusion protein. Implications for therapeutic and vaccine strategies:

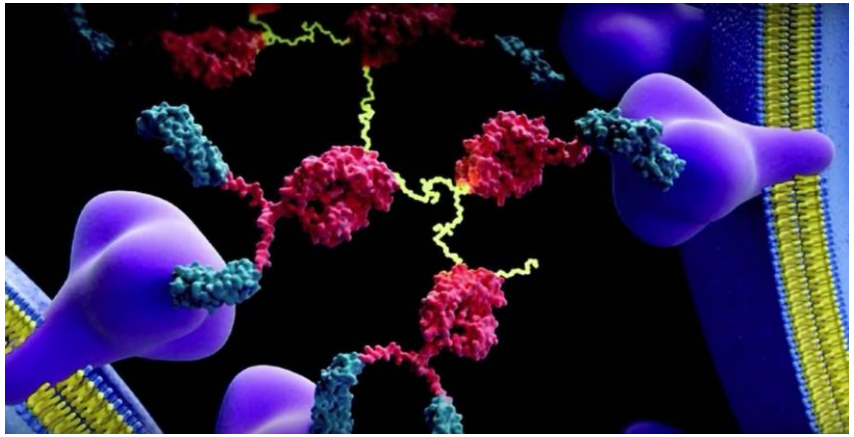
Arthos, J.; Cicala, C et al., JBC 2002;277(13):11, 456-464 [Open Access]



FLASK

FIBERCELL

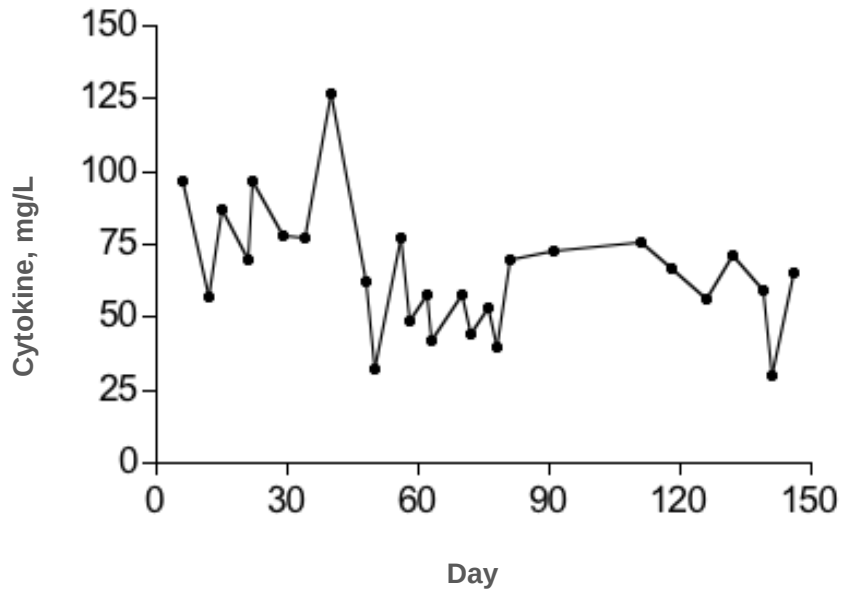
CryoET image of hexamerized IgG

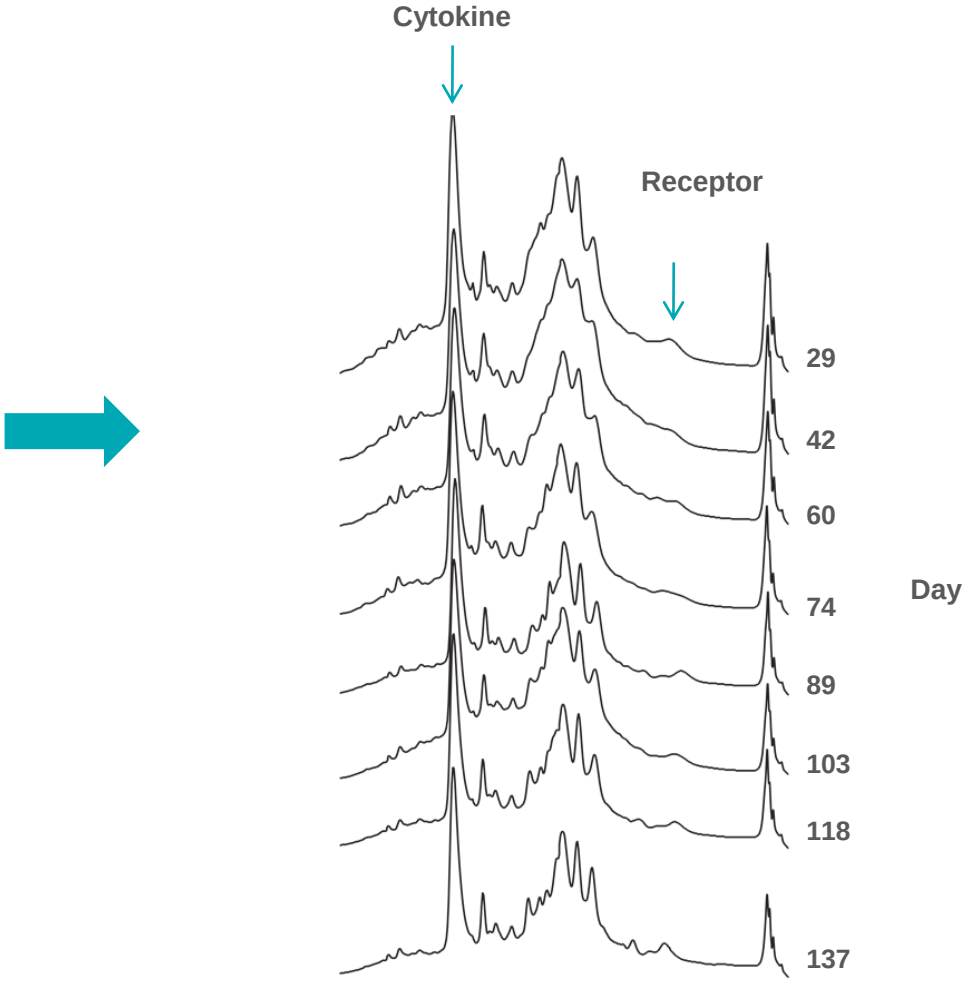


Characterization and Favorable in Vivo Properties of Heterodimeric Soluble IL-15•IL-15Rα Cytokine Compared to IL-15 Monomer:

Chertova, E. et al.; The Journal of Biological Chemistry 2013 288: 18093-18103. [Open Access]

HFBR Production - mg/L



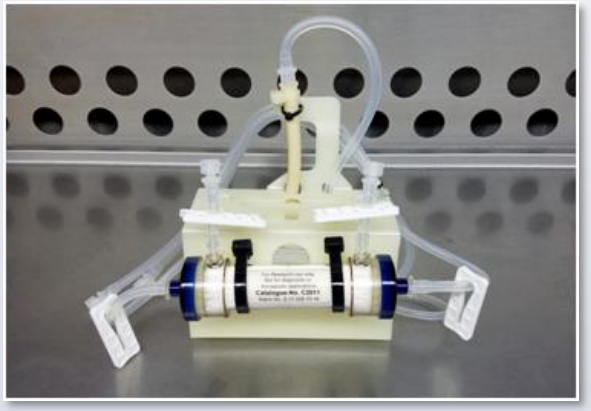


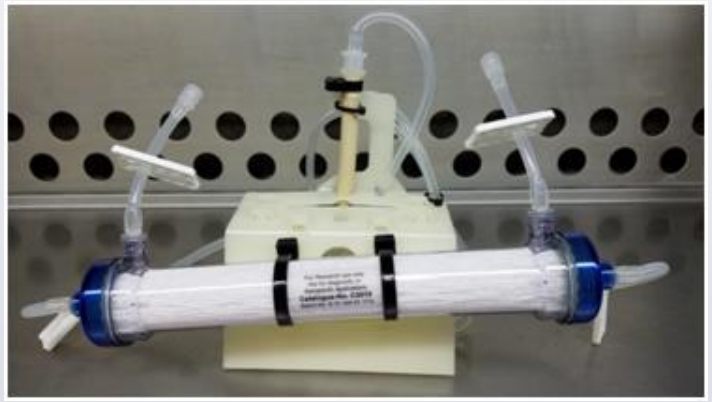
RP-HPLC Analysis

Serum Free Harvests

Typical Productivity for Recombinant Protein

- 20 ml cartridge – 2 mg per day
- 70 ml cartridge – 8 mg per day





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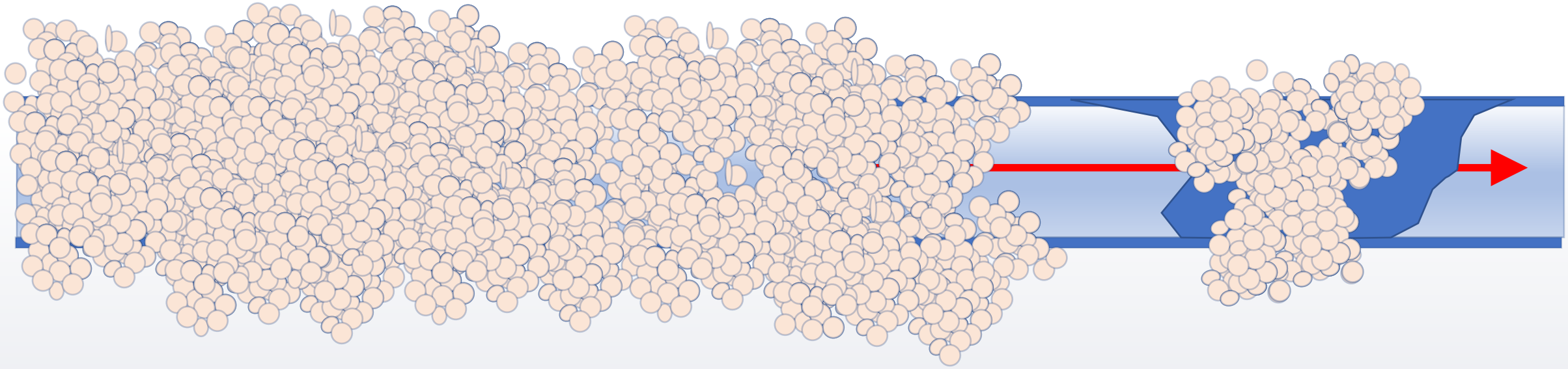
3D culture environment



..simplification of culture media possible

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CDM-HD mentioned in EV-related publications

Osteosarcoma-Derived Extracellular Vesicles Induce Lung Fibroblast Reprogramming: Alekhya Mazumdar et al. IJMS 21 (15) 2020 [**open access**]

AL-PHA beads: bioplastic-based protease biosensors for global health applicationsRichard Kelwick et al. [**open access**]…

Hypoxia-induced tumor exosomes promote M2-like macrophage polarization of infiltrating myeloid cells and microRNA-mediated metabolic shift: Park, JE et al.; Oncogene (2019) [**abstract**]

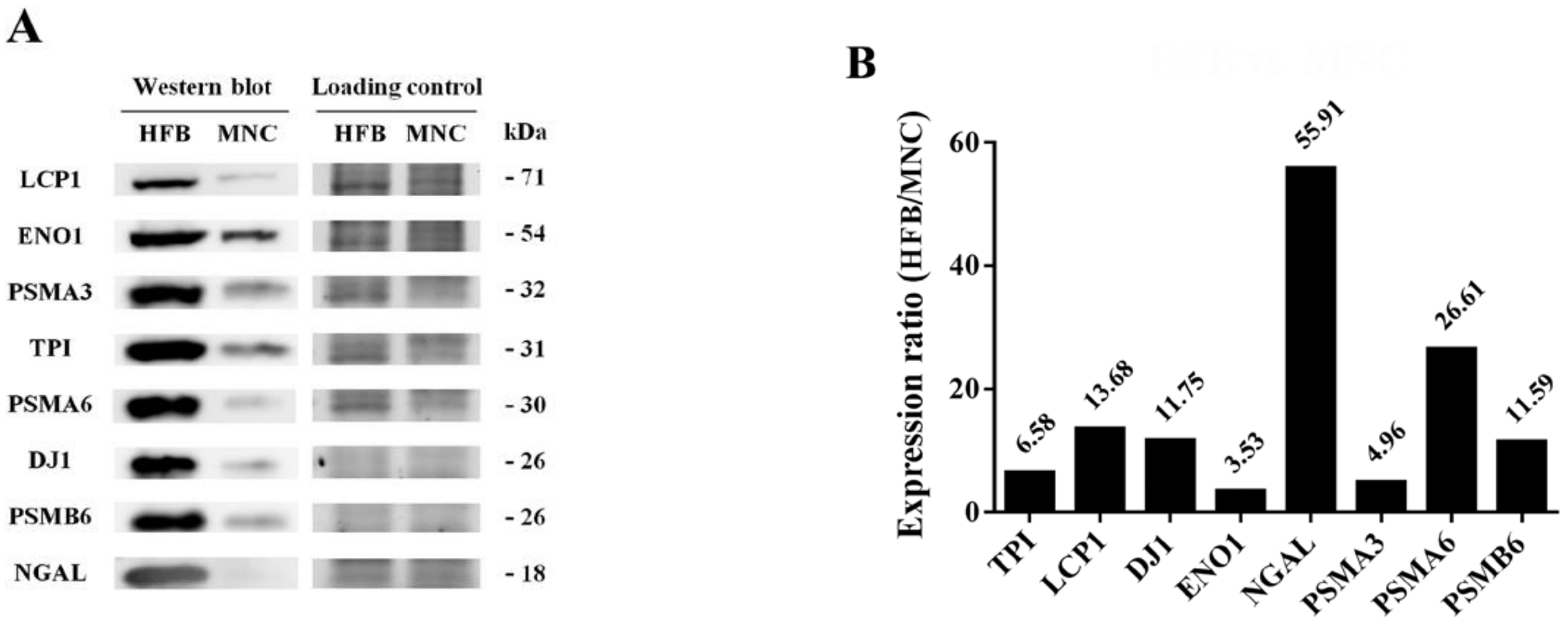
Large-scale preparation of extracellular vesicles enriched with specific micro-RNA: Yoo, KW et al. ; Tissue Engineering. 2018 [**abstract**]

Efficient production and enhanced tumor delivery of engineered extracellular vesicles: Watson DC et al.; Biomaterials 105, 2016: 195-205 [**open access**]

Secretomic profiling of cells from hollow fiber bioreactor reveals PSMA3 as a potential **cholangiocarcinoma** biomarker:

Verathamjamras,C. et al; Int J Oncol. 2017 51(1):269-280.

Monolayer culture (MNC) vs. HFB



95 proteins > 2 fold changes

- BLUE: 20S proteasome core
- RED: cell cycle
- GREEN: metabolic pathway

Hollow fibre vs monolayer culture

Verathamjamras,C. et al; Int J Oncol. 2017 51(1):269-280.

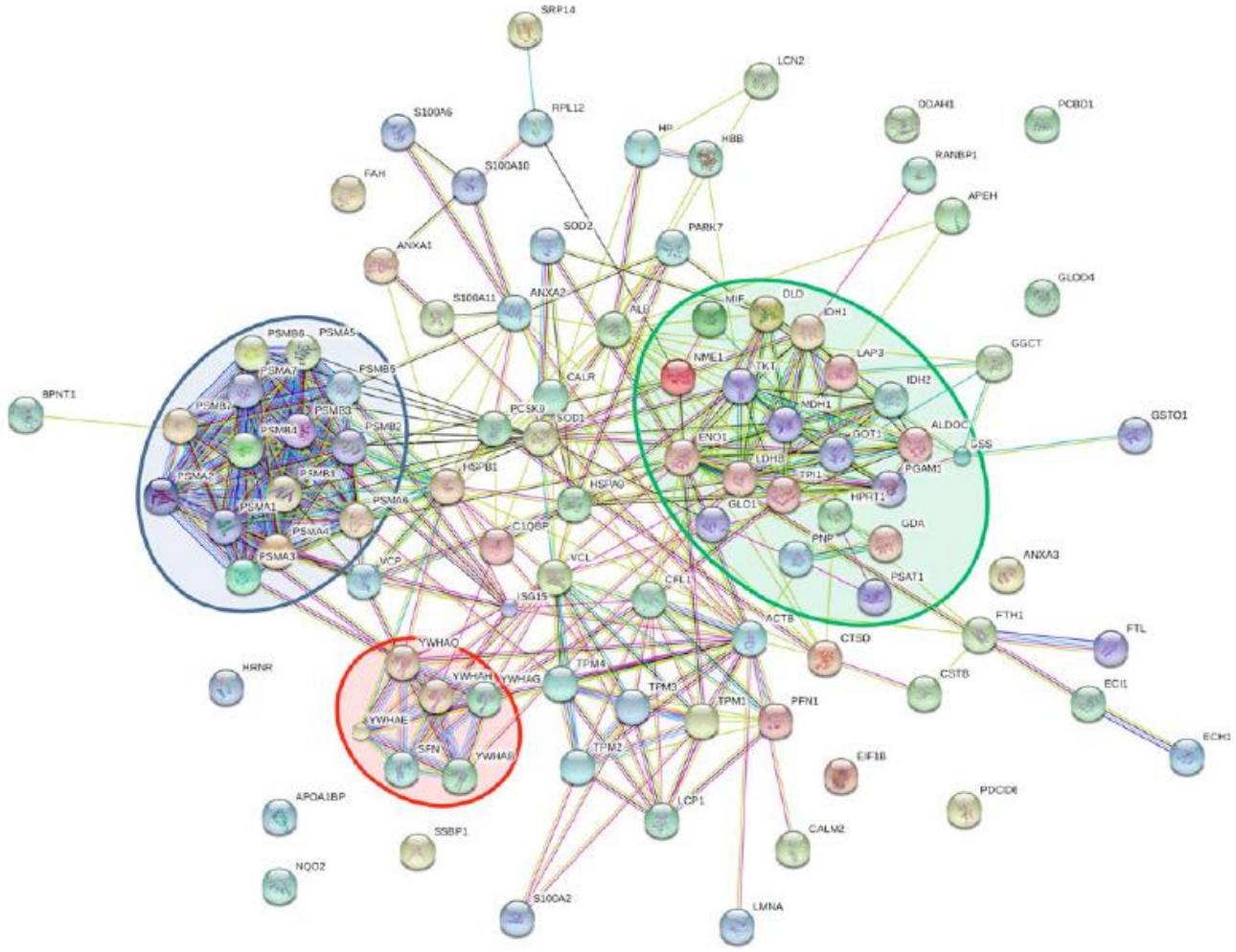
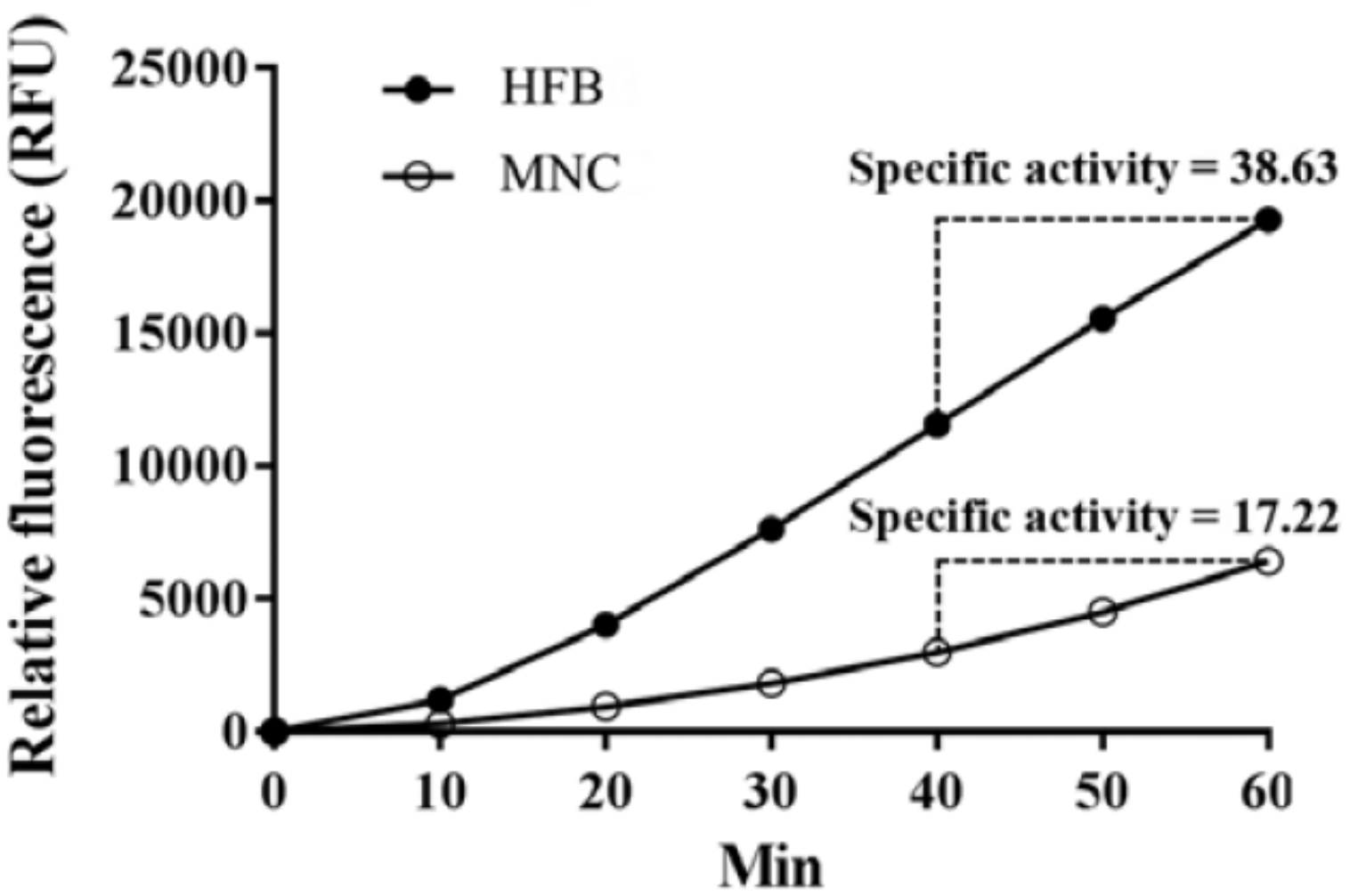


Figure 3. STRING analysis of 95 proteins significantly increased in HFB system as compared to MNC system (>2-fold changes). The interaction networks can be divided into three major clusters, namely proteins related in metabolic pathways (green background), 20S proteasome core subunits (blue background), and cell cycle pathways (red background).

Relative proteasome activity

Hollow fibre vs monolayer culture

Verathamjamras,C. et al; Int J Oncol. 2017 51(1):269-280.



EV related publications

2018 - 2020

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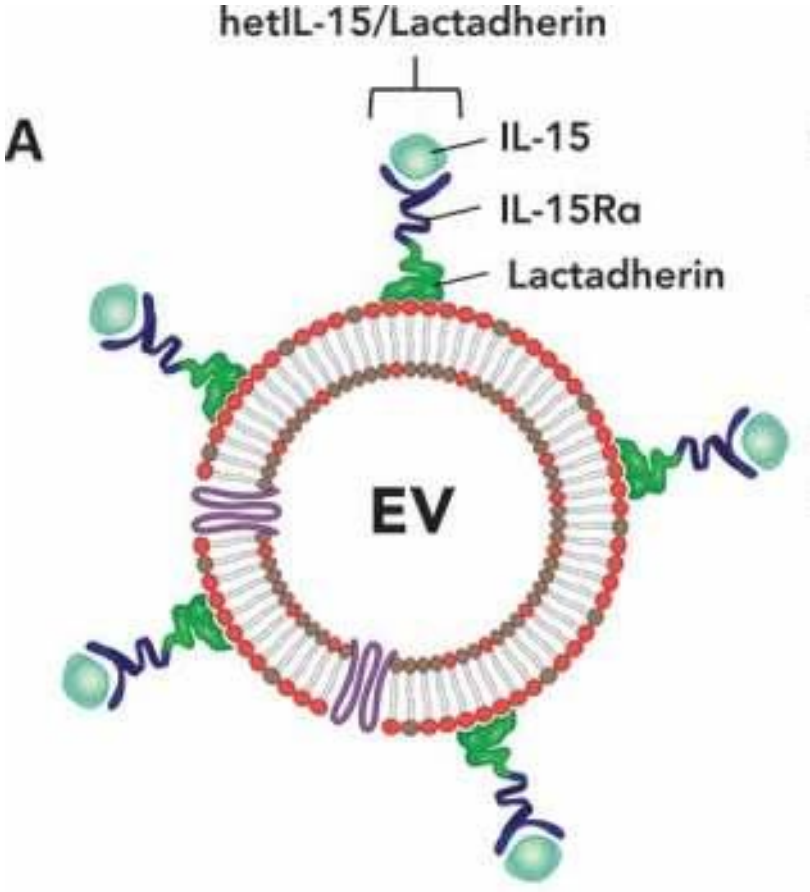
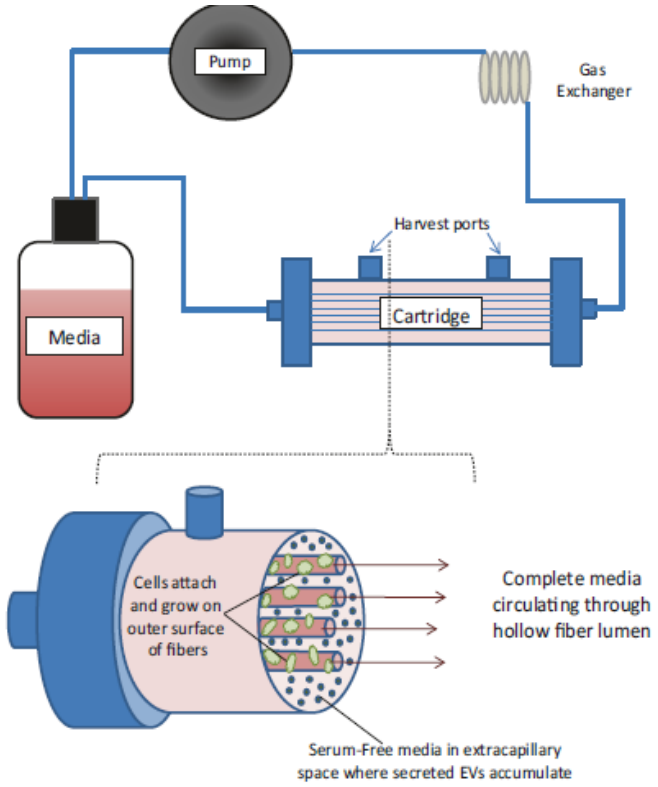
Scalable, cGMP-compatible purification of extracellular vesicles carrying bioactive human heterodimeric IL-15/lactadherin complexes:
Watson,D.C. et al.; J Extracell Vesicles. 2018 Feb 28;7(1) [open acccess]

…We cultured recombinant HEK293 cells expressing hetIL-15 in a hollow-fibre bioreactor with serum-free media….and found that yield of EV was consistently >40-fold higher than conventional tissue culture flasks….

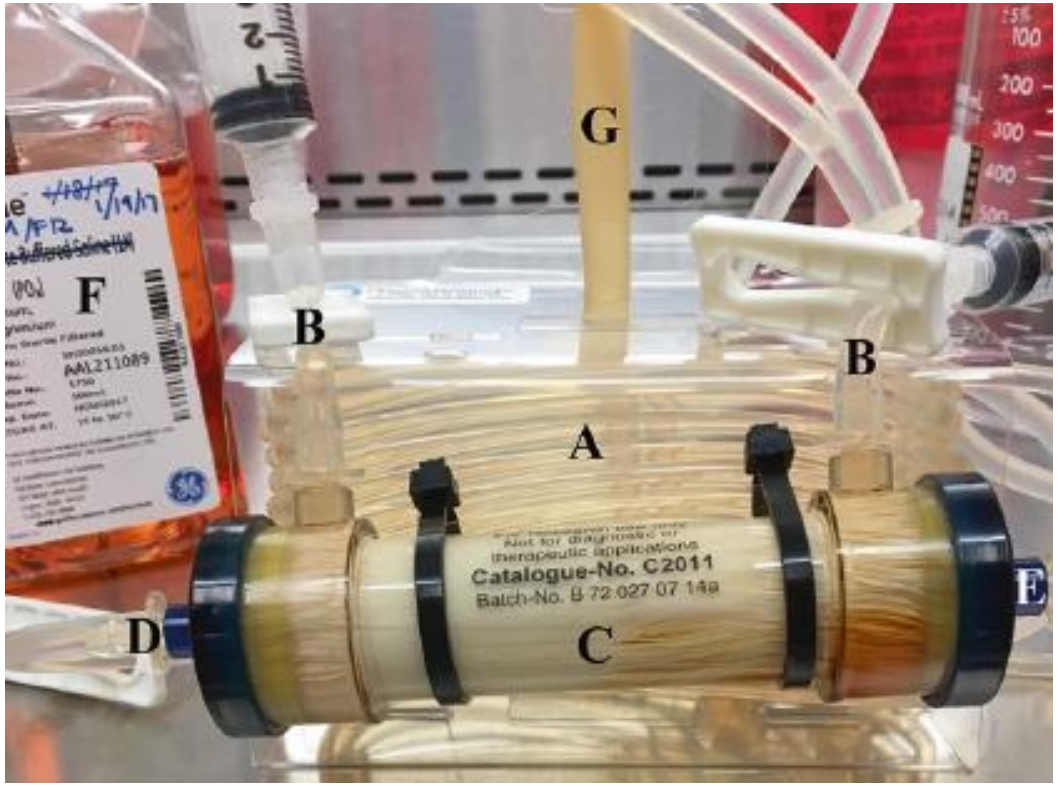


Author provides his complete protocol for EVs harvesting from FiberCell System



Use of a Hollow Fiber Bioreactor to Collect Extracellular Vesicles from Cells in Culture:

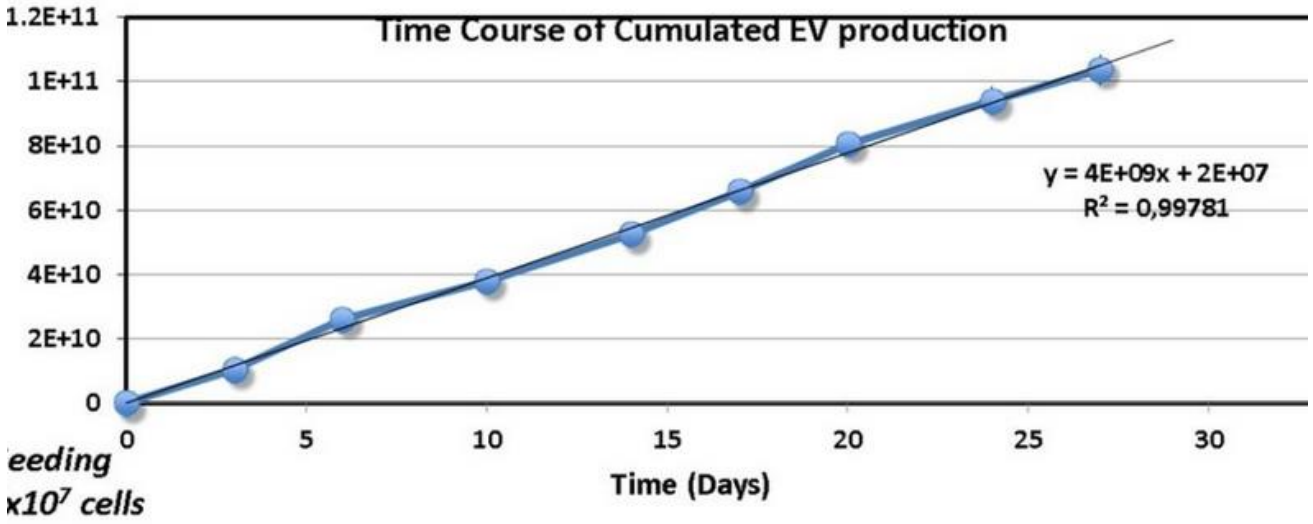
Yan IK et al.; Methods Mol Biol. 2018;1740:35-41



EVs from cbMSCs (INSERM U1197)

"We have now performed three experiments with cord blood MSC. In all three cases, the production of extracellular vesicles was linear with time up to 4 or 5 weeks of culture."

NTA Analysis of EV produced by Bone-marrow MSC in FiberCell Duet Bioreactor – Small Cartridge C2025D

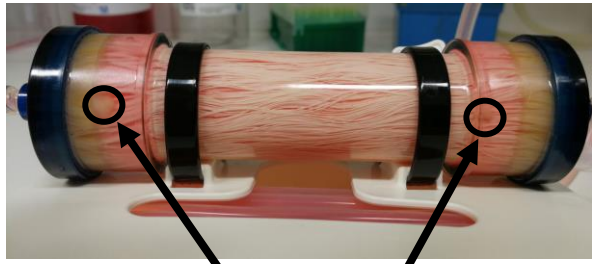


EVs produced using FiberCell C2025D Hollow Fibre Cartridge

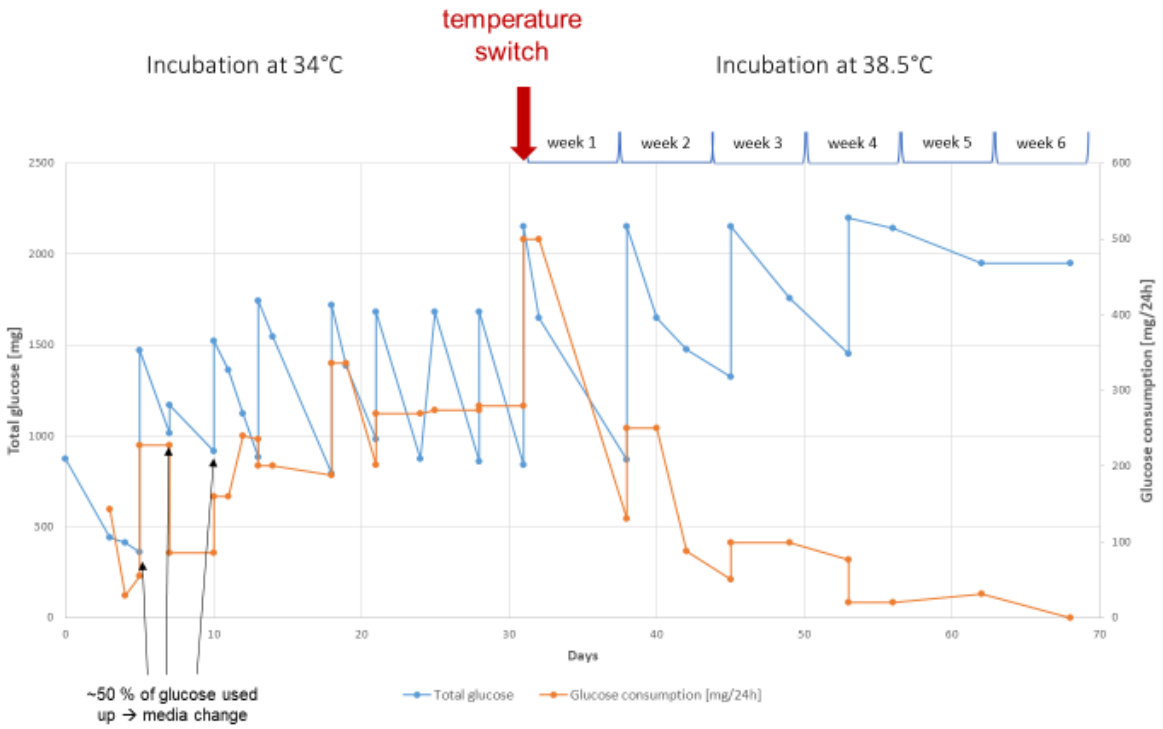
Data courtesy of Philippe Mauduit, INSERM U1197, V

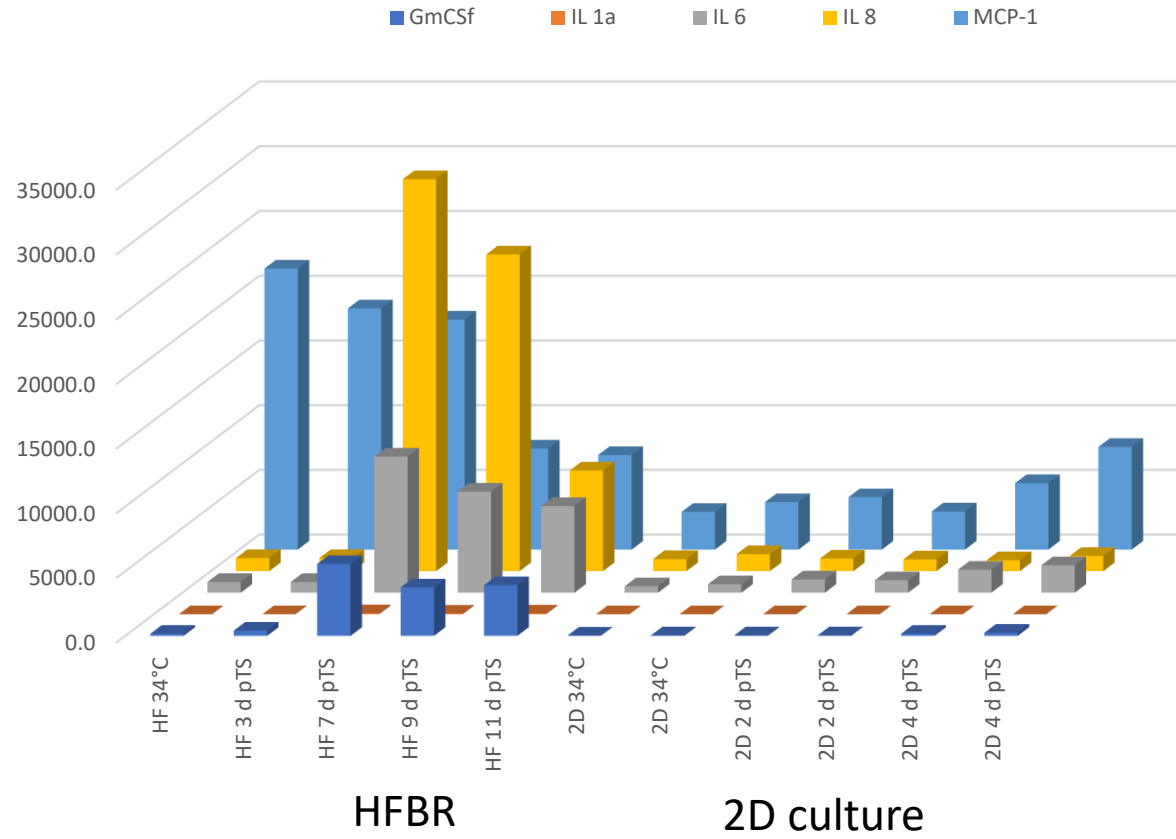
Hollow Fibre Reactor for large scale production of senescent cell derived extracellular vesicles

Elena Stelzer, Vera Pils, Johannes Grillari, Institut für Biotechnologie, 27.10.2018



Cells growing in 3D nodules





HFBR

2D culture

Large-scale preparation of extracellular vesicles enriched with specific micro-RNA:

Yoo, KW et al. ; Tissue Engineering. 2018

The hollow fiber bioreactor system allows high-density culture of EV-producing cells, and the 20 kDa cutoff of the fiber allows nutrients and waste products to pass through but retains EVs (40–1000nm in diameter, molecular weight >500 kDa). We grew the HEK293-mir-133a, HEK293-mir-202, and HEK293-mir-NT cells in the system for >30 days, and **collected 50mL of EV-enriched supernatant every 1 or 2 days.** We discontinued the cultures only because we collected enough supernatant.

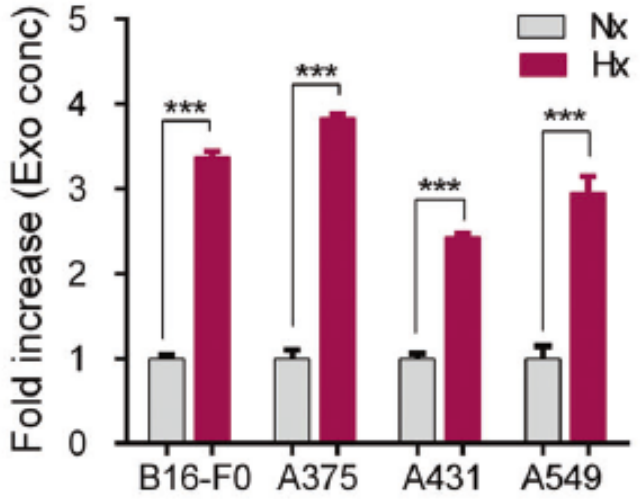
Compared with supernatants of conventional 2D cultures, expression of miR-133a-3p and miR-202-5p in the supernatants collected from the bioreactors contained 4.3-fold more miR-133a-3p or miR-202-5p in EVs.

EMAIL STATEMENT FROM AUTHOR:
"Yes, we do believe that the 3D culture conditions provide a more physiological environment. In addition, the number of cells cultured in a 3D environment is much greater than a 2D culture. We believe that this is a great system to obtain high density extracellular vesicles."
- Kyung Yoo,

Hypoxia-induced tumor exosomes promote M2-like macrophage polarization of infiltrating myeloid cells and microRNA-mediated metabolic shift:

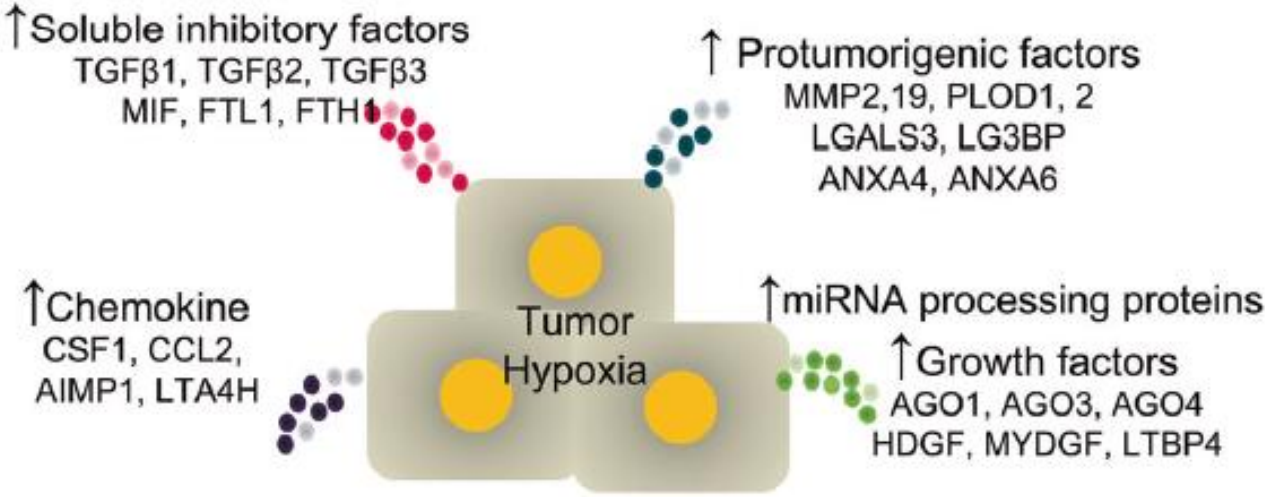
Park, JE et al.; Oncogene (2019) [abstract]

Exosome concentration increase



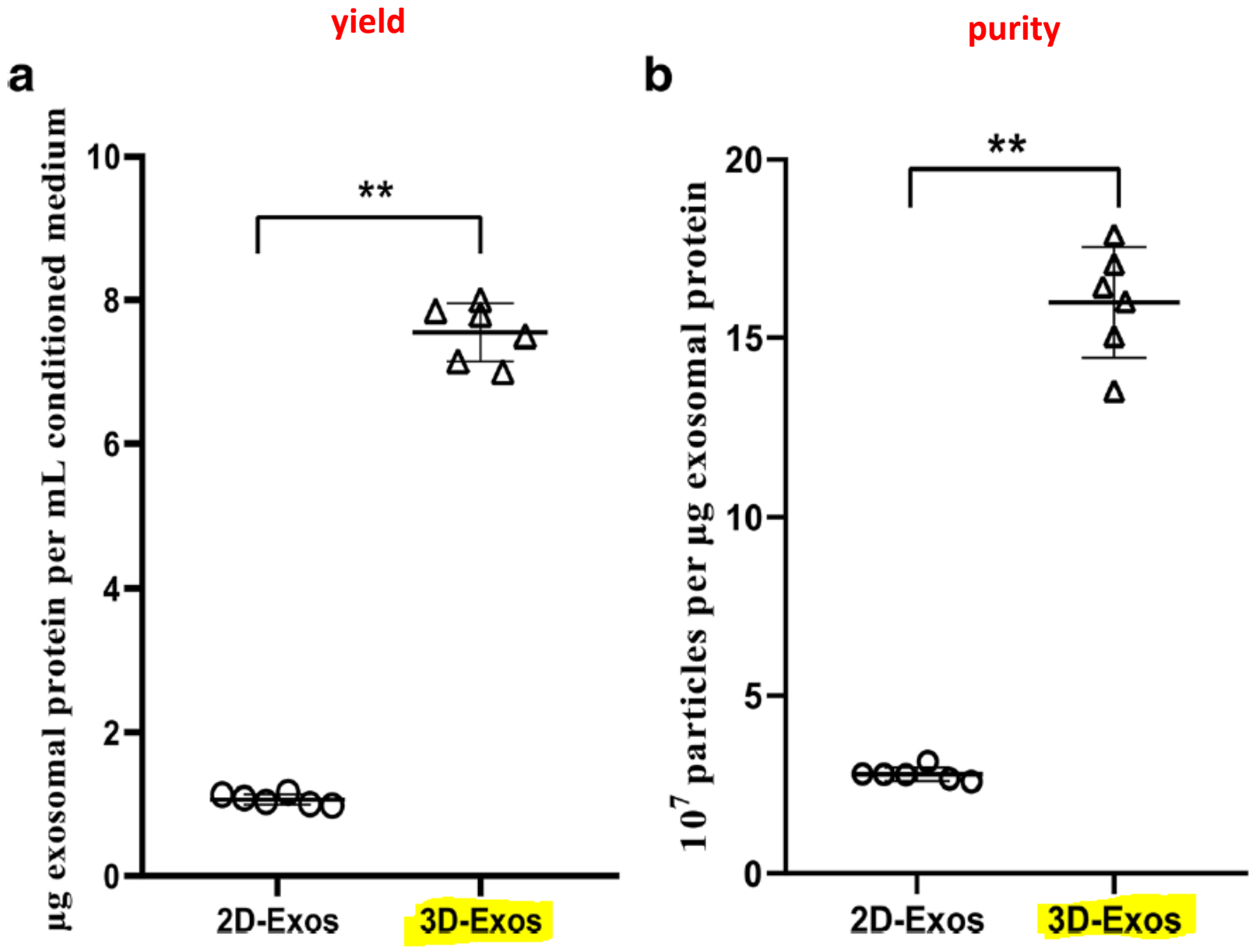
"We used a hollow fiber culture system to generate an in-vivo like 3D environment"

Hypoxic B16 cells- exosome proteins that were enriched



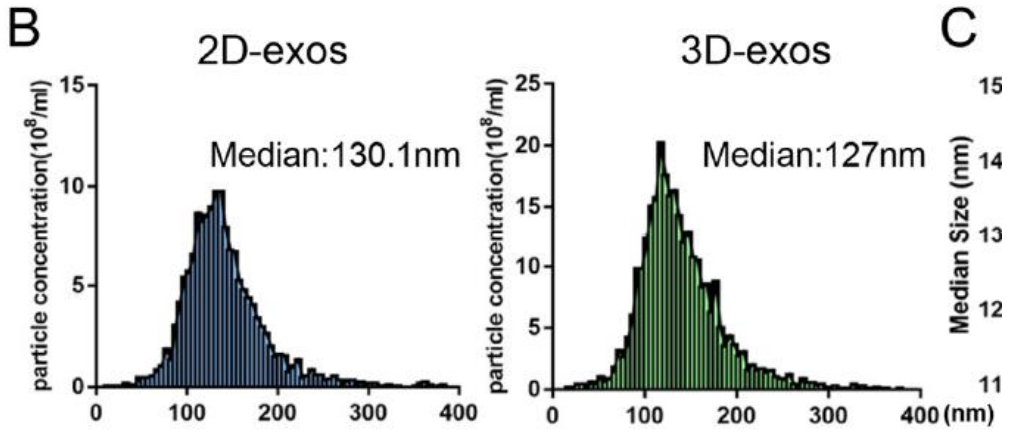
Exosomes produced from 3D cultures of umbilical cord mesenchymal stem cells in a hollow-fiber bioreactor show improved osteochondral regeneration activity:

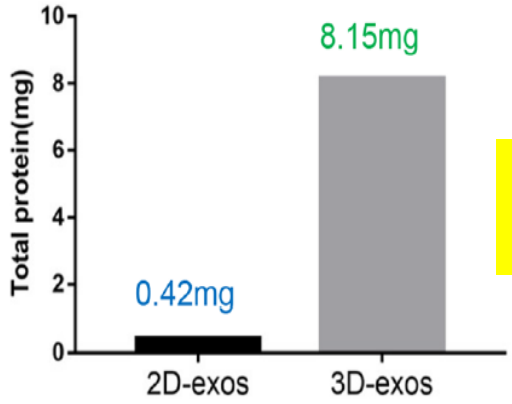
Litao Yan & Xing Wu; Cell Biol Toxico (Dec 09 2019) [open access]



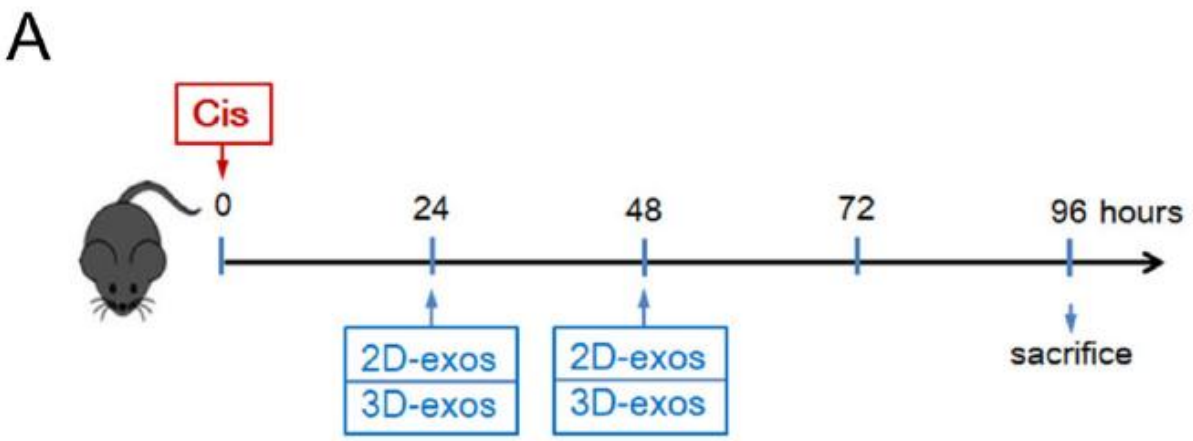
Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury

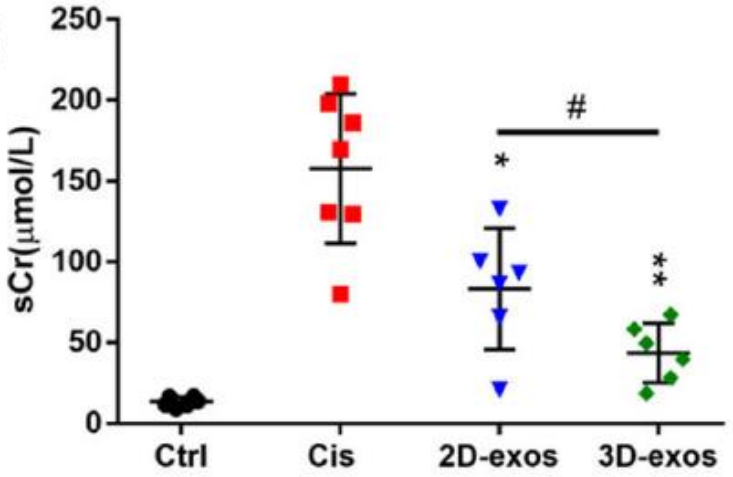
Cao et al. Stem Cell Research & Therapy (2020) 11:206 [open access]





Total exos output
52 days



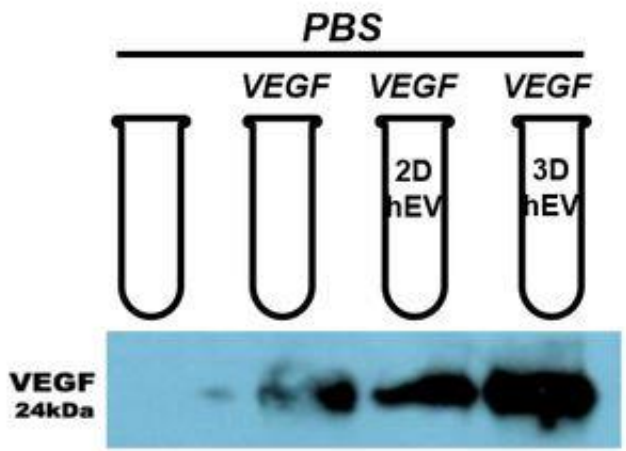


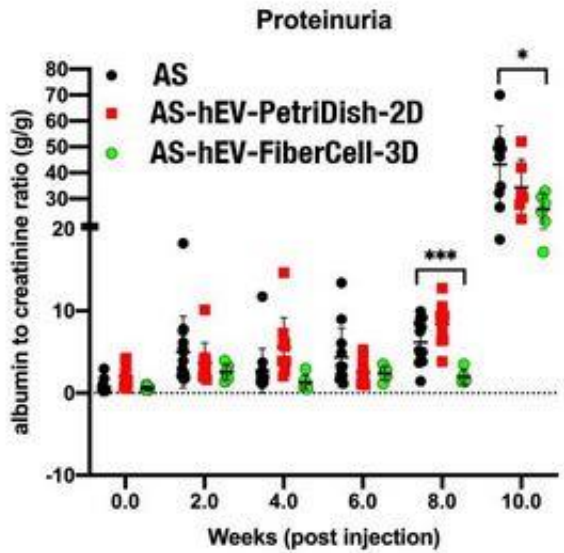
Enhanced efficacy

EVs produced in 3-D hollow fiber bioreactor are equivalent to 2-D EVs

EV clinical transition is limited by scale-up of EVs production. Hollow fiber bioreactor (HFBR) can support culture of large numbers of cells at high densities and can produce large numbers of EVs.

Amniotic fluid stem cell EVs produced in a 3-D hollow fiber bioreactor are biochemically and functionally equivalent to petri dish 2-D EVs, with yields that can support clinical scale in a cGMP compatible system





Continuous culture of 10^9 to 10^{11} cells

***in vivo* like conditions**

Very high yields

Simplifies purification

Avoid serum EV contamination

Clinical scale production possible for a modest size lab

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Last Slide

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