Dissecting Spent Media to Elucidate Components and Mechanisms that Affect Cell Culture Performance

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Background and Project Overview

Overall Goals: Identify growth-inhibiting and growth-promoting extracellular components in CHO-culture spent media. **UD work emphasizes impact of EVs and their components & collection of perfusion spent media.**

Extracellular vesicles (EVs) are native mediators of cell-to-cell communication

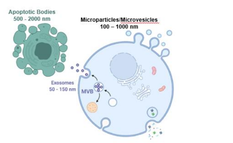
Nano-sized membrane particles formed from cell membranes that facilitate the transfer of biomolecules: small RNAs (largely miRNAs, **miR**s), lipids, proteins, mRNAs and some DNA

Types of EVs:

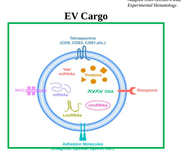
Exosomes – endosomal biogenesis; 50-150 nm

Microparticles – plasma membrane biogenesis; 100-1000 nm

Apoptotic bodies – formed during apoptosis; >500 nm



Adapted from Cocozzo-Pheas, J & Abraham, S.A. (2021) *Experimental Hematology*.



Tao, S.C., & Guo, S.C. (2020) *Cell Commun Signal.*

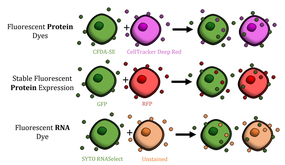
Using CHO-K1 VRC01, this study aims:

1) Develop protocols and study the role of EVs in Batch, Fed-Batch, and Perfusion cultures
2) Identify EV and cell components in fractionated spent media
3) Identify differences between media from batch, fed-batch and perfusion cultures

Native EVs facilitate massive exchange of proteins and nucleic acids among CHO cells under normal & stress conditions

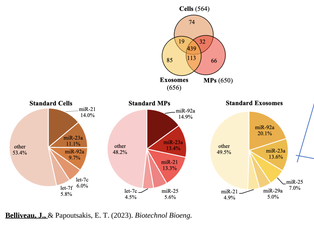
Fluorescent tagging of EV components

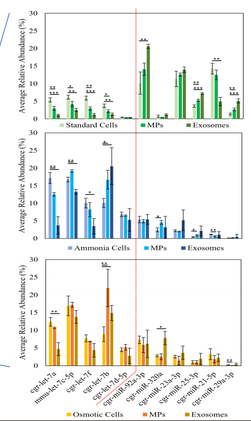
- Coculture CHO cells expressing different fluorescent proteins or stained with different dyes
- Use of Microscopy and Flow Cytometry (FC) enables visualization of EV-mediated cellular material exchange among cells in culture



Micro-RNA (miRNA; miR) profiling

- 5 miRNAs represent 50% of total identified miRNA EV content
- miR EV content is not the same as parent cell miR content: sorting process is involved
- Stress conditions change miRNA abundances in both cells and EVs

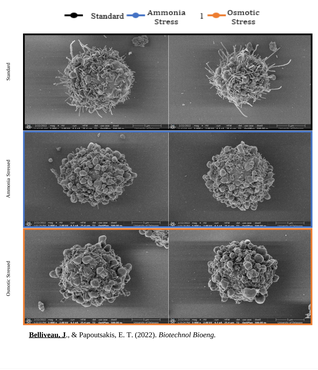




Belliveau, J., & Papoutsakis, E. T. (2023). *Biotechnol Bioeng.*

Scanning electron microscopy (SEM)

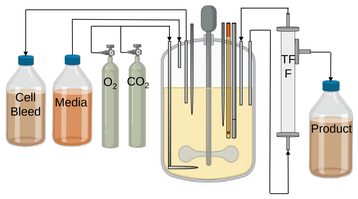
- Shows EV-like structures and exchange events on the cell surface
- Bolus dose of 9 mM ammonium chloride (**Ammonia stress**) or 60 mM sodium chloride (**Osmotic stress**) at the start of culture
- Stress enriched miRs regulate critical cell functions affecting stress response



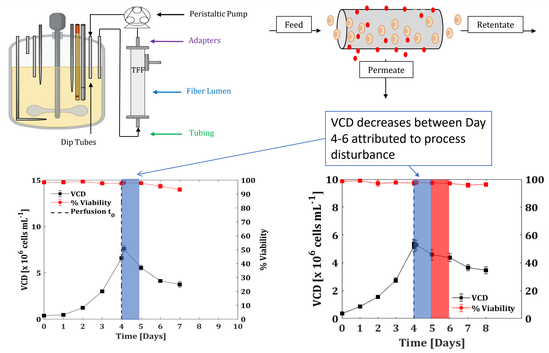
Belliveau, J., & Papoutsakis, E. T. (2022). *Biotechnol Bioeng.*

Perfusion Experiments to Collect Spent Media

1. Perfusion Bioreactor Experiments



Base case perfusion media formulation:
HyClone ActiPro basal media
3% (v/v) HyClone Cell Boost 7a
0.3% (v/v) HyClone Cell Boost 7b
Supplemental glucose targeting $C_{Glucose}$=9 $g\ L^{-1}$



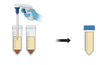
Day 4-5; representing culture media at the end of batch phase and beginning of perfusion

Day 5-6; representing culture media after one day of perfusion operation

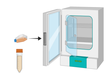
(continued) 2. Pseudo-perfusion process

Step 1: Centrifuge cell culture at 180g for 5 minutes to pellet cells.

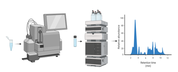
Step 2: Media exchange via supernatant removal and cell resuspension in fresh, pre-warmed media.



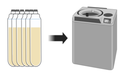
Step 3: Pool supernatant across biological duplicates to harvest EVs.



Step 4: 24-hour incubation with orbital agitation at 250 rpm for spin tubes and 150 rpm for T-flasks.

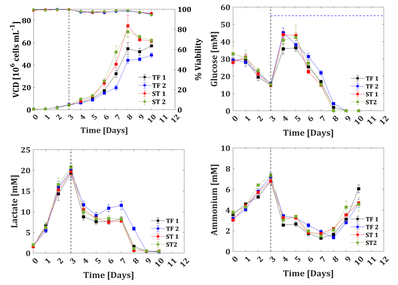


Step 5: Off-line metabolite measurements via HPLC (amino acids and titer) and YSI Biochemistry Analyzer (glucose, lactate, ammonia).

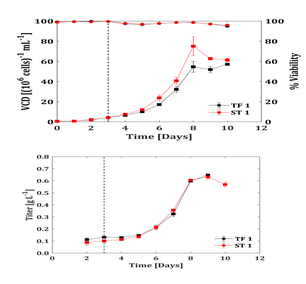


Step 6: Harvest EVs (microparticles and exosomes) via differential ultracentrifugation.

Pseudo-perfusion (ST1,2) simulates well real perfusion profiles (TF1,2)

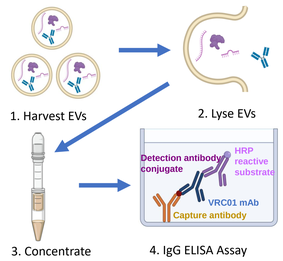


Pseudo-perfusion titer profiles without a cell bleed establish a productivity baseline



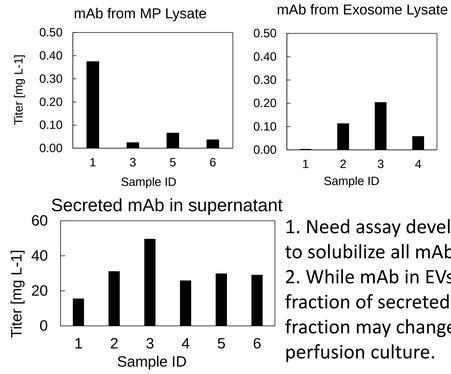
ELISA assay detects recombinant mAb in EVs from high cell density pseudo-perfusion cultures

Experimental Workflow



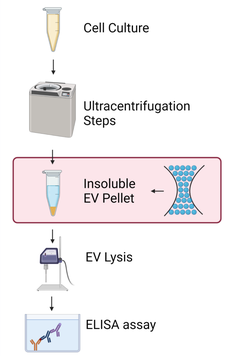
Sample ID	Pooled Supernatant	Volume [mL]	Experimental Condition
1	Days 5-6	30	High glucose T-flask duplicates
2	Days 7-8	30	High glucose T-flask duplicates
3	Days 9-10	30	High glucose T-flask duplicates
4	Days 6-7	30	Low glucose T-flask duplicates
5	Days 8-9	30	Low glucose T-flask duplicates
6	Days 5-10	30	High glucose spin tube duplicates

Preliminary Results (Malinov)

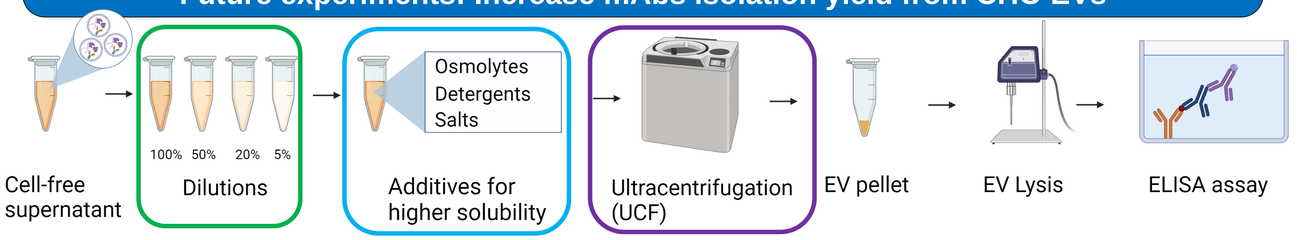


1. Need assay development to solubilize all mAb in EVs
2. While mAb in EVs is a small fraction of secreted mAb, that fraction may change in perfusion culture.

Potential Bottleneck



Future experiments: Increase mAbs isolation yield from CHO EVs



1: Dilute EV solution to mitigate aggregation

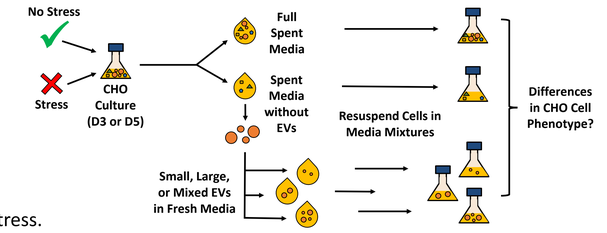
2: Supplement with additives before UCF to increase protein solubility

3: Adjust UCF speed and times to reduce stress

Future Experiments on the Role of EVs in CHO Cell Culture

What effects of spent medium result from EV bioactivity?

1. Remove spent medium from CHO cultures on days 3 and 5.
2. Separate small and large EVs (exosomes and microparticles).
3. Resuspend cells in the following mixtures:
   a. Full spent medium
   b. Spent medium without EVs
   c. Small EVs in fresh medium
   d. Large EVs in fresh medium
   e. All EVs in fresh medium
   f. Fresh medium only
4. Test VCC, apoptosis, and glucose/glutamine metabolism to day 12.
5. Repeat for cultures exposed to ammonia, lactic acid, and oxidative stress.



References & Acknowledgements

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[3] Clincke, M. F. et al. (2013). Biotechnology progress, 29(3), 754-767.
[4] Belliveau,J.,& Papoutsakis, E.T(2022) Biotechnol Bioeng.
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