DEVELOPMENT OF AN INTEGRATED BIOPROCESS FOR HIGH-DENSITY CULTURE OF IMMUNOTHERAPEUTIC NK-92 CELLS

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BACKGROUND AND MOTIVATION

- NK-92 cells exhibit cytotoxicity against a variety of cancer cell lines, including leukemia's, lymphoma's, and malignant melanoma's. This activity has lead to several small clinical trials as an anti-cancer immunotherapy, with larger multi-centre Phase II trials planned.

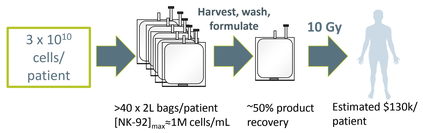

Figure 1. The current manufacturing strategy for NK-92 based on static bag culture is expensive and not scalable for large patient numbers. Translation into closed dynamic culture systems will allow for more efficient expansion, lower labour and infrastructure costs, and a clear path for scale-up. Culture intensification combined with more efficient downstream processing are identified as targets for process improvement.

- The current production strategy relies on static culture techniques, resulting in large culture volumes and high cost. Volume and cost are further inflated by inefficient harvest and wash, with cell losses as high as 50%.
- Our ***GOAL*** is to enable cost-effective large-scale manufacture of clinical grade NK-92 cells.

BIOSEP ACOUSTIC FILTRATION FOR INTEGRATED PERFUSION CULTURE, HARVEST AND WASH

Figure 2. The MiniBio stirred tank reactor (STR) system from Applkon with a BioSep acoustic filter unit. STRs provide a closed, dynamically-controlled, and scalable system for cell expansion. The BioSep (right) generates standing acoustic waves to capture cells in suspension, allowing for the removal of spent media. This technology has been extensively demonstrated for perfusion culture but could also be useful for cell harvest, wash, and concentration.

AIMS

- Expand NK-92 cells in stirred-tank bioreactors (STR) with BioSep-based perfusion feeding to enable an intensified culture process that is readily scaled for commercial manufacture.
- Integrate BioSep separation technology for efficient downstream harvesting and wash.

OBJECTIVES

- Translate NK-92 expansion protocol into stirred tank reactors.
- Define BioSep parameters for optimal separation efficiency.
- Intensify cultures for higher cell densities through optimized perfusion feeding strategies.
- Demonstrate high efficiency harvest and wash with minimal process time.
- Integrate culture and downstream processes.
- Scale process to produce clinically relevant cell quantities.

NK92 TRANSLATION TO STIRRED TANK REACTORS – FED-BATCH FEEDING

- NK-92 inoculation (100 mL @ 0.5M cells/mL) cultured for six days, with media doubled every 2nd day.
- STR resulted in greater cell expansion compared to static culture.

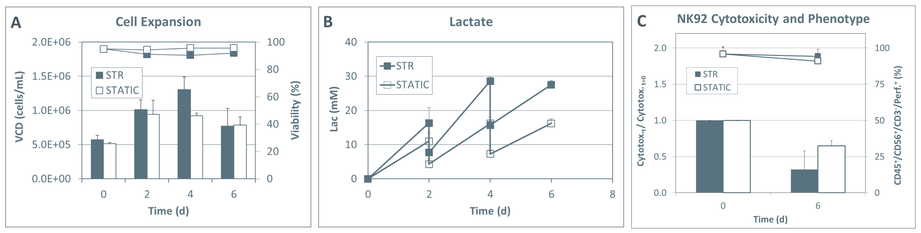

Figure 3. A) NK-92s expand faster in STRs compared to static, but both systems show a decrease in viable cell density (VCD) at day 6. **B)** Lactate accumulates in cultures and may explain growth inhibition. This effect is higher in STR due to higher cell densities. Glucose was not limiting (data not presented). **C)** Although the NK phenotype is maintained, cells cultured in STRs loose potency. (n=3)

- Successful transition of static culture to STR .
- Accumulation of lactate may limit cell expansion/potency, rationale for perfusion culture.

BIOSEP CELL CONCENTRATION

- NK-92 cells in suspension (150 mL, 0.5M cells/mL) were concentrated using the Mini BioSep at flow rates up to 1.2 mL/min.
- High separation efficiency (SE) was observed, with minimal loss of cells in the waste stream.

$$\text{SE}\,(\%) = \left(1 - \frac{\text{VCD harvest}}{\text{VCD bulk}}\right) \cdot \mathbf{100}$$

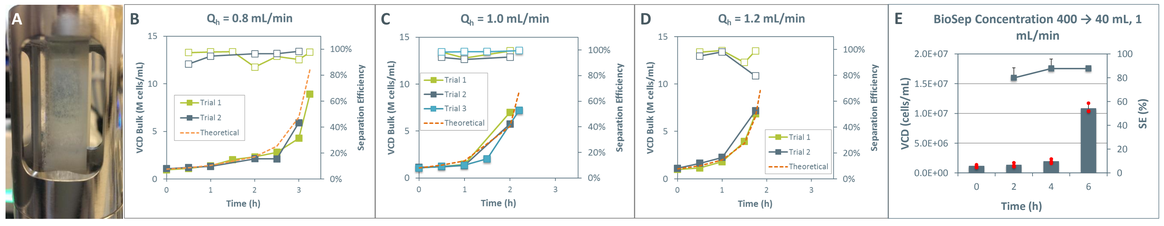

Figure 4. A) NK-92 cells are captured in the BioSep acoustic chamber while media is removed. Cells are returned to the bioreactor periodically. **B-D)** Cell density plots during Mini BioSep concentration of 150 mL cultures at flow rates of 0.8, 1.0, and 1.2 mL/min respectively. Separation efficiency is maintained above 90% even at high flow rates. **E)** BioSep-mediated concentration is efficient when scaled for 400 mL cultures.

BIOSEP settings	
Power	1 W
$t_{harvest}$	10 min
$t_{back\text{-}flush}$	5s or **10s**

- The BioSep demonstrates very good separation efficiency across cell concentrations from 0.5 to 10 M cells/mL at flow rates suitable for perfusion.
- At these flow-rates, process time for harvest and wash would take multiple hours. Options include using larger model BioSep or modify device design.

STR CULTURES WITH BIOSEP PERFUSION - PRELIMINARY RESULTS

- NK-92 inoculation (100 mL @ 0.5M cells/mL) cultured for 8 days with three different perfusion feeding profiles to investigate effect of volume exchange rate on maximum achievable cell density and metabolite profile.
  - One culture volume equivalent per day (1D) compared to two ramped profiles (Ramp A and Ramp B).
- BioSep operated at 0.5 W at flow rates to continuously extract spent media as fresh media added at matching flow rate.

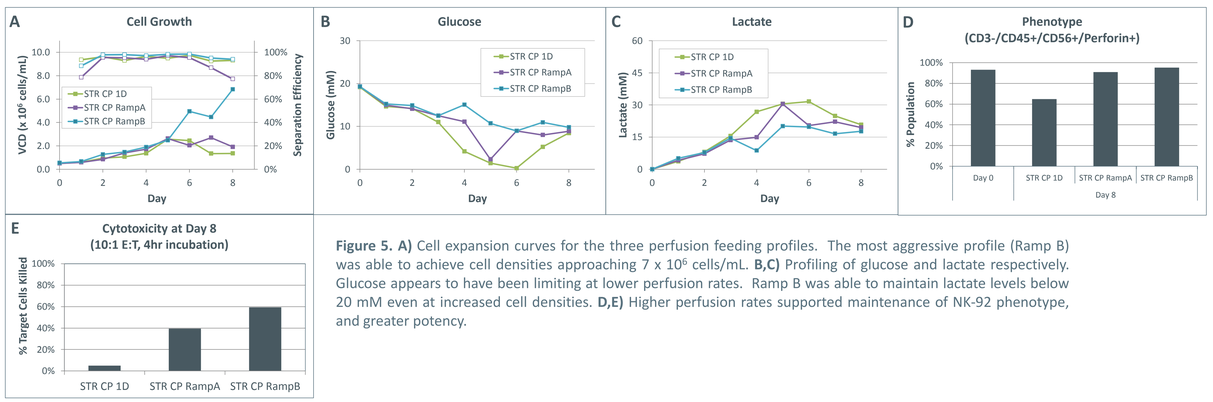

Figure 5. A) Cell expansion curves for the three perfusion feeding profiles. The most aggressive profile (Ramp B) was able to achieve cell densities approaching 7 x 10^6 cells/mL. **B,C)** Profiling of glucose and lactate respectively. Glucose appears to have been limiting at lower perfusion rates. Ramp B was able to maintain lactate levels below 20 mM even at increased cell densities. **D,E)** Higher perfusion rates supported maintenance of NK-92 phenotype, and greater potency.

- Successful intensification with viable cell density of > 5M cells/mL.
- Media exchange has beneficial effects to maintaining NK-92 phenotype and potency.

BIOSEP HARVEST AND WASH – PRELIMINARY RESULTS

- NK-92 cells in suspension (400 mL, 3M cells/mL) harvested using 10 L/d BioSep.
- Achieved separation efficiencies > 90% at flow rates up to 4 mL/min.
- Some minor modifications to be addressed to improve wash efficiency (e.g. minimize concentrated volume).

- Proof-of-concept for single acoustic filtration-based device for perfusion, harvest and wash

Significance and on-going work

- NK-92 cells can be cultured in STR and can expand more efficiently compared to static cultures.
- The BioSep acoustic filter can remove spent media while maintaining NK-92s in the bioreactor with high separation efficiency.
- Perfusion culture using the BioSep can greatly intensify cultures compared to traditional fed-batch feeding. Feeding strategies must be adjusted as cell densities rise.
- SonoSep technology is promising for downstream harvest and wash and warrants further investigation.