Demonstrating Scalable T-Cell Expansion in Stirred-Tank Bioreactors Using Ambr® 15 Cell Culture

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Introduction
This study used the Ambr® 15 Cell Culture bioreactor system to accomplish two goals:
- Demonstrating T-cell proliferation in stirred tank bioreactors
- Establishing key parameters of stirred-tank bioreactor cultures of T-cells

Two perfusion mimic schemes, where 50% of the medium volume was exchanged every other day and 35% was exchanged every third day, were tested. The low perfusion scheme involved an addition of 12.1 mg/day of glucose, and high perfusion corresponded to an addition of 16.9 mg/day.

This proof-of-concept study outlines a scaled-down T-cell culture method that is representative of current full-scale manufacturing methods, and has the potential to outperform current technologies when combined with true perfusion and medium exchange rates of 100% per day normally seen in manufacturing environments.

Methods
This study was conducted with negatively selected CD3+ cells rested overnight in cell media of X-VIVO 10, 5% human AB serum, and 100 U/mL IL-2 prior to the addition of 1 x 106 cells/mL. Culturing in the Ambr® 15 Cell Culture bioreactor system resulted in approximately a 2.5-fold and 3-fold increase in total viable cell yield over the static cultures for the low and high perfusion rates, respectively. The compounded medium exchanges, however, resulted in < 20% reduction in total yield due to cells removed during the perfusion mimic. Greater T-cell yields would be expected in a fully developed bioreactor system equipped to perform true perfusion, without cell losses.

Establishing Key Parameters of Stirred-Tank Bioreactor Cultures

Bioreactor Conditions
- Agitation settings resulted in a specific power input of 5.04 W/m
- pH settings were evaluated Ambr® 15 Cell Culture bioreactors operated without media exchanges prior to the main experiment.

Bioreactor Conditions
- The low perfusion scheme involved an addition of 12.1 mg/day of glucose, and high perfusion corresponded to an addition of 16.9 mg/day.
- Lactate concentration asymptotically approaches 7.6 mg/dL. Cell death correlated with lactate concentrations above 7.0 mg/dL.

Conclusion
Impeller-driven mass transfer resulted in a 3-fold increase in cell number compared to the static, batch-fed controls at the conclusion of a thirteen-day culture period. Cell growth arrest between days nine and thirteen controls at the conclusion of a thirteen-day culture period. This study was conducted with negatively selected CD3+ cells rested overnight in cell media of X-VIVO 10, 5% human AB serum, and 100 U/mL IL-2 prior to the addition of 1 x 106 cells/mL. Culturing in the Ambr® 15 Cell Culture bioreactor system resulted in approximately a 2.5-fold and 3-fold increase in total viable cell yield over the static cultures for the low and high perfusion rates, respectively. The compounded medium exchanges, however, resulted in < 20% reduction in total yield due to cells removed during the perfusion mimic. Greater T-cell yields would be expected in a fully developed bioreactor system equipped to perform true perfusion, without cell losses.

Future Work
- A key next step is to directly compare this scale-down model with existing manufacturing unit operations for T-cell therapies.
- Limit the two-day lag phase experienced after seeding.
- Analyze the effect of impeller agitation on the transition from central T-cells to effector cells.
- Explore a re-stimulation unit step after Day 9.
- Determine the effect on COGS expenditures.

Figure 1.

Demonstrating T-Cell Proliferation in Stirred-Tank Bioreactors

CellPhenotyping
- There is no significant change to the resultant T-cell identities in an impeller agitated system compared to a static culture.
- All conditions showed an expected fecundity to the CD4+ T-helper phenotype post-culture.

Culture Expansion
- The Ambr® 15 Cell Culture bioreactor system showed statistically significant improvements over static cultures with high perfusion rates (p < 0.0055; t-stat = 4.59), but the low perfusion rates did not result in statistically significant improvements (p = 0.6; t-stat = 0.53).
- Bioreactor cultures with high perfusion rates resulted in four times the number of cells as batch-fed cultures.
- A 3.5x daily media exchange supported a cell density 8.8x 106 cells/mL.
- Growth rates were negative for the first two culture days in the Ambr® 15 Cell Culture bioreactors.

Figure 2.

Percent of Total Cells
- Starting Material
- Batch-Fed
- Low Perfusion
- High Perfusion

Figure 3.

Lactate Concentration Over Time

Figure 4.

The change in key metabolite concentrations over the course of the thirteen-day culture (N=3)