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

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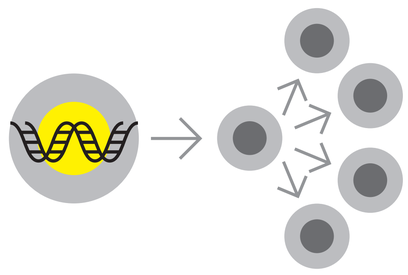
## Introduction

This study used the Ambr® 15 Cell Culture bioreactor system to accomplish two goals:

- Demonstrating T-cell proliferation in stirred-tank bioreactors
- Establish key parameters of stirred-tank bioreactor cultures of T-cells

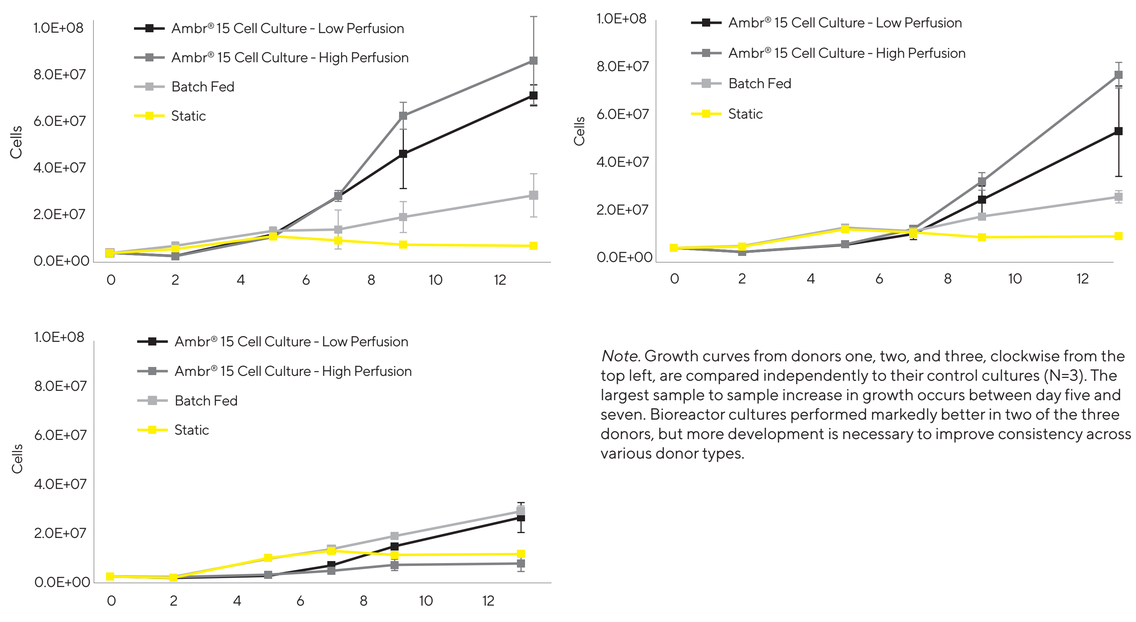
Two perfusion mimic schemes, where 50% of the medium volume was exchanged every other day and 35% of the medium was exchanged each day, were tested against batch-fed cultures in T-flasks maintained at 1x10$^6$ cells/mL. Culturing in the Ambr® 15 Cell Culture microbioreactor system resulted in approximately 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.

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.



## Demonstrating T-Cell Proliferation in Stirred-Tank Bioreactors

**Figure 1.**




*Note.* Growth curves from donors one, two, and three, clockwise from the top left, are compared independently to their control cultures (N=3). The largest sample to sample increase in growth occurs between day five and seven. Bioreactor cultures performed markedly better in two of the three donors, but more development is necessary to improve consistency across various donor types.

### Cell Phenotyping

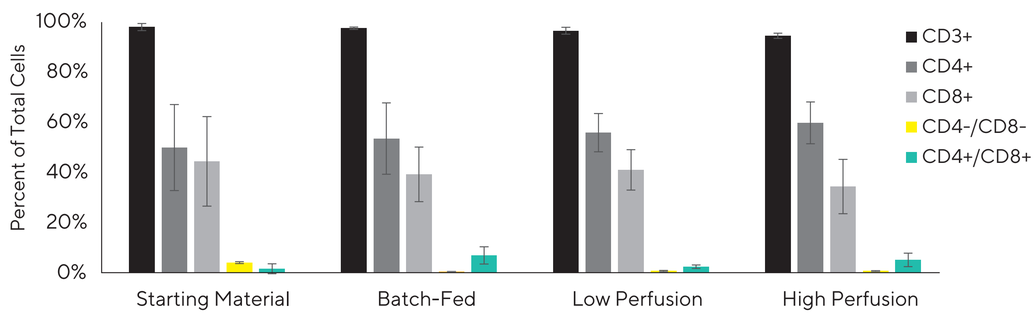
- There is no significant change to the resultant T-cell identities in an impeller agitated system compared to a static culture.
- All conditions showed an expected favorability to the CD4+ T-helper phenotype post culture.

### Culture Expansion

- The Ambr® 15 Cell Culture bioreactor system showed statistically significant improvements over static cultures with high perfusion rates (p = 0.0059, t Stat = 4.59), but the low perfusion rates did not result in statistically significant improvements (p=0.61, t Stat = 0.537).
- Bioreactor cultures with high perfusion rates resulted in four times the number of cells as batch-fed cultures
- A 35% daily media exchange supported a cell density 8.83 x 10$^6$ cells/mL.
- Growth rates were negative for the first two culture days in the Ambr® 15 Cell Culture bioreactors.

**Figure 2.**

*Common T-cell phenotypes visualized to show no changes across the culture conditions (N=9).*




## 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 splitting the cells into their individual cultures. The cells were incubated with CD3/CD28 activation beads at a ratio of 3:1.

Static cultures were plated with 4 mL of cell suspension at 1 x 10$^6$ cells/mL and maintained at 4 mL for the length of the study. The batch-fed cultures were seeded with 4 mL of prepped cell suspension at 1 x 106 cells/mL, and these cultures were expanded to maintain a cell concentration of 1 x 10$^6$ cells/mL after each sampling event. The Ambr® 15 cultures were inoculated with 5 x 10$^6$ cells and cultured in a total volume of 10 mL, and the vessels were static for the first four days of culture. Half of the vessels had a 50% media exchange performed every other day and in the other culture station a 35% media exchange was performed each day. Analytics were performed using the NC-200, the FACSCanto II, and the Cedex Bio Analyzer for cell counting, cell phenotyping, and nutrient and metabolite analysis respectively. The cultures were analyzed for CD3 cell purity and relative change to the subpopulations between the bioreactor and static cultures. Perfused media was tested for glucose, glutamine, total protein, lactate, and ammonia concentrations.

## Establishing Key Parameters of Stirred-Tank Bioreactor Cultures

### Bioreactor Conditions

- Agitation settings resulted in a specific power input of 5.04 W/m$^3$.
- 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.

**Figure 3.**

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

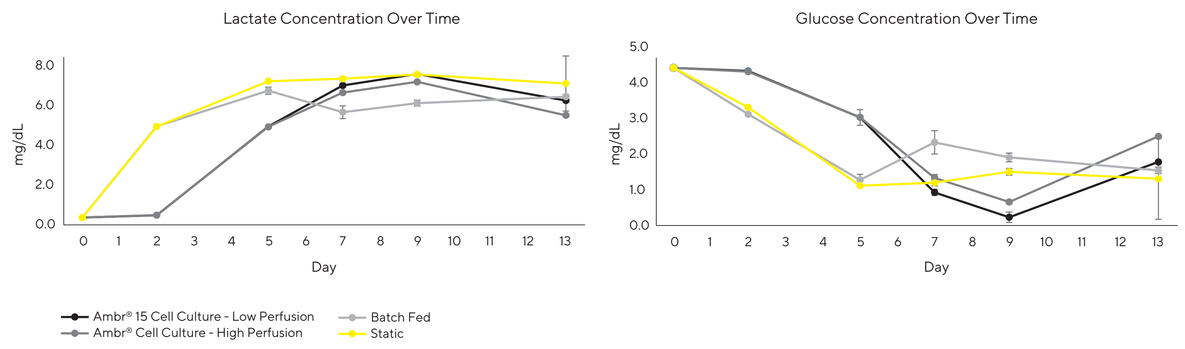



**Figure 4.**

*Average fold expansion for un-perfused Ambr®15 Cell Culture bioreactor cultures in two pH conditions (N=12)*

| | Fold Increase pH 7.2 | pH 7.4 |
|---|---|---|
| Day 5 | 1.9 | 1.6 |
| Day 7 | 3.0 | 2.5 |
| Day 8 | 3.0 | 2.5 |

## 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 should be investigated controlling for metabolite levels using higher perfusion rates. Another area of future study is into the lag phase experienced after initial seeding into the Ambr® 15 Cell Culture microbioreactors. This observation should be compared to other agitated bioreactor systems as well as static expansion systems to gauge the source of this cell loss. Aspects of the culture that should be considered are the planar density of cells (cells/cm ), the gas transfer gradient which would be directly correlated to the depth of media, and mass transfer limitations. Furthering the knowledge of human primary T-cell expansion is pertinent to develop more robust manufacturing protocols for emerging therapies, and the results of this study demonstrate that a stirred-tank bioreactor system could be utilized for more efficacious T-cell expansion and reduced cost of goods with shortened culture periods that would be relevant for patient-specific processes and emerging off-the-shelf T-cell therapies.

## 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 effecter cells
- Explore a re-stimulation unit step after Day 9.
- Determine the effect on COGS expenditures