Pharma&Biotech

Developing Patient-specific Cell Therapy Manufacturing Processes: Reducing CoGs While Maintaining Quality Parameters

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1. Abstract

Patient-specific cellular therapies hold tremendous potential for being able to treat life threatening diseases where there are currently no cures. However, these therapies have several challenges that may impede the path to commercialization which will delay them from becoming widely available. One of several challenges for this nascent field are the complex and expensive manufacturing processes required to produce these new therapies under the quality requirements of pharmaceuticals. Lonza is helping therapeutic companies address these challenges through efficient and effective process development programs aimed at streamlining their manufacturing processes to simplify them and reduce the cost of goods (CoGs), all while maintaining the quality parameters and important biology that are required for these unique, living cell-based products to maintain their therapeutic efficacy. In this set of studies we optimize a model T-cell expansion protocol for yield and phenotype, and demonstrate large scale processes capable of producing billions of T-cells within 10 days among various platforms. We have also developed a closed system harvest in which can undergo a volume reduction as well as a buffer exchange resulting in a sterile product suitable for long term storage without loss of viability

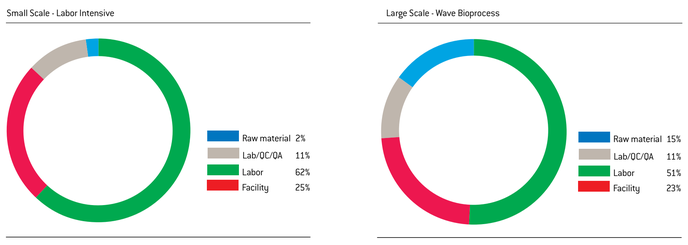


Figure 1. **Small scale processes are laden with labor while larger scale processes are burden with materials.** Schematic of cost drivers in early and late stage T-cell processes. In early, typically "open" cultures, the main cost driver is labor. Larger cultures utilizing automation and additional media have transitioned to raw materials as the major cost driver. Different types of processes have different cost drivers, emphasizing the importance to model processes and examine costs prior to the process development campaign.

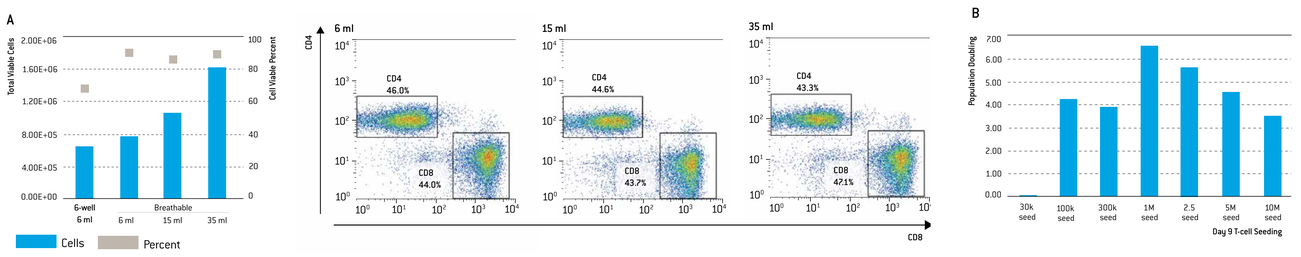
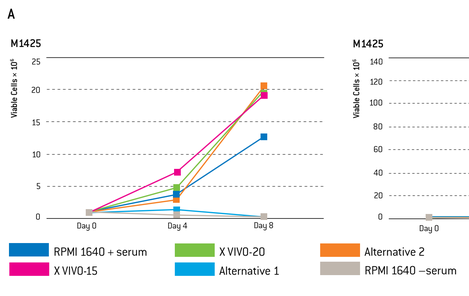
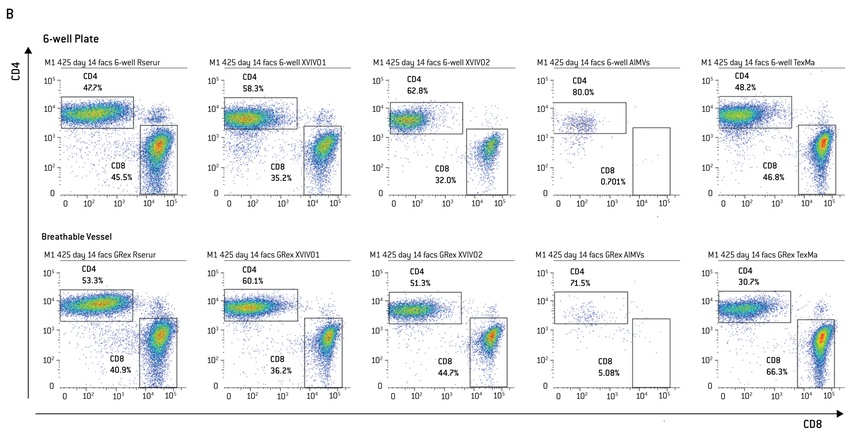


Figure 2. **T-cell Model expansion optimized for 35 ml and 1 M seeding density offers maximum population doublings.** In establishing a model platform for T-cell expansion, several parameters were examined in order to optimize growth and phenotypic consistency. (A) PBMCs were stimulated with CD3/CD28 Dynabeads® in both 6-well plates (open system) and a breathable bioreactor (closed system) with varying media volumes. After 14 days of culture, increased media volume in a closed system resulted in increased cell density, while maintaining viability (left) and phenotypic consistency (right). (B) Increasing numbers of T-cells were cultured and expanded with CD3/CD28 Dynabeads® in the closed system containing 35 ml media. The closed system supports relatively large scale expansion (~100-fold) during 9 days of culture with minimal labor and no additional costs, while maintaining high viability and CD4/CD8 consistency (data not shown).





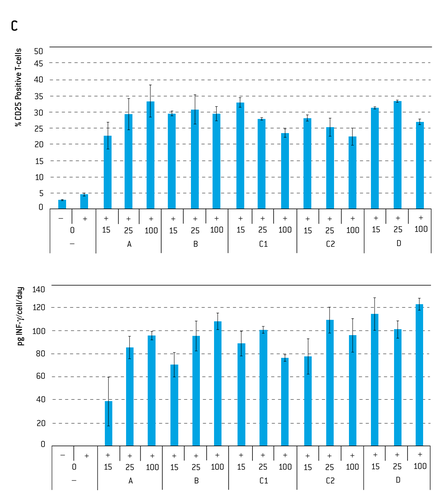


Figure 3. **Serum-free media outperforms serum-based media and maintains phenotype, with secondary suppliers for cytokines.** T-cells (1x10⁶) were stimulated with CD3/CD28 Dynabeads® in both 6-well plates (5 ml) and a closed system breathable vessel (35 ml) with different media. The serum-free X-VIVO media supported the largest expansion of T-cells, comparable to RPMI supplemented with human serum in both open and closed systems and outperforming several serum-free media alternatives (A) as well as maintaining high viability (data not shown). The CD4/CD8 phenotype was consistent among the different media conditions (B). Multiple suppliers of GMP-grade IL-2 were also examined for their ability to support T-cell growth (data not shown), expression of the CD25 activation marker and IFN-g secretion demonstrating comparable levels of CD25 expression and IFN-g production (C).

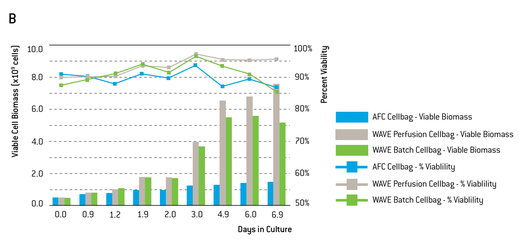


Figure 4. **Large scale T-cell expansion is optimal in perfusion Wave Cellbags.** In order to compare large scale expansion of T-cells in closed systems, starter cultures were initiated with CD3/CD28 Dynabeads® in mini-bioreactors for 5-6 days (A). From these cultures, 5x10⁸ T-cells were seeded into 1 L of X-VIVO media and transferred into a WAVE perfusion bag, WAVE bag (no perfusion), or an AFC 290-C bag. The cultures were monitored daily for cell count and viability (B). The AFC bag culture generated a ~3-fold expansion of cells in 7 days, with decreasing viability as the culture progressed. The WAVE culture bags gave a 5-7 fold expansion during the 7 days of culture with the perfused culture having a greater growth. The benefit of perfusion on the WAVE cultures is demonstrated with the ~95% viability of the culture after 7 days, while the batch culture viability rapidly dropped to ~85%.

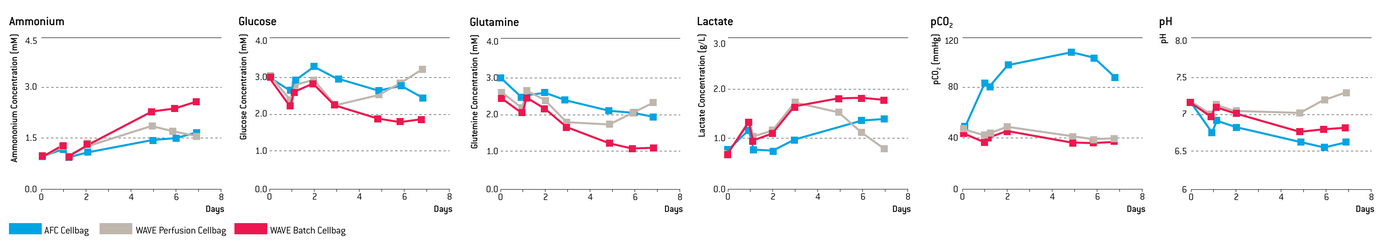


Figure 5. **Metabolite monitoring can drive perfusion feed schedule.** WAVE bag 1 liter and AFC bag cultures from Figure 4 were monitored daily for glucose, glutamine, pH, lactate, and ammonium using a NOVA Flex Analyzer. Perfusion was enabled after 3 days of culture on one of the cultures and the rate was adjusted based on daily readings. Metabolic waste and byproducts accumulated with time in the AFC bag culture and the batch WAVE culture, whereas they remained stable or decreased with the perfused WAVE culture. Glucose and glutamine decreased with the AFC bag and batch WAVE cultures, however remained at adequate levels with the perfused WAVE cultures.

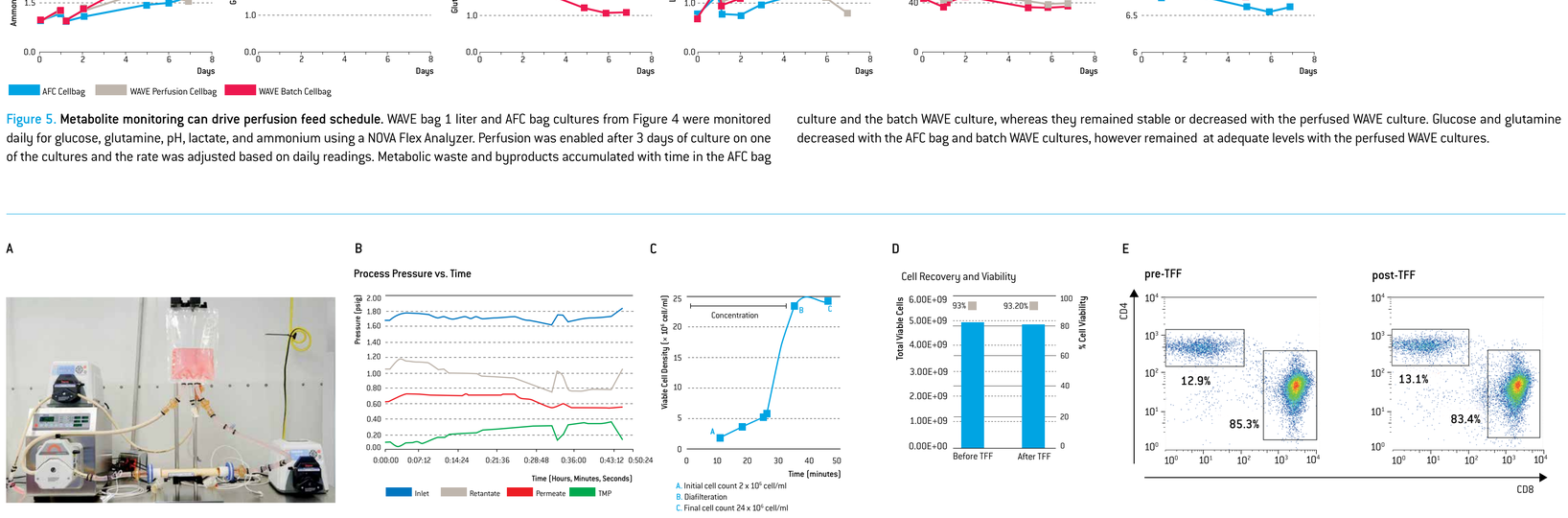


Figure 6. **Tangential Flow Filtration can reduce volume while maintaining viability.** The closed WAVE culture bag system from Figure 4 can be connected to a hollow fiber tangential flow filtration system (TFF) using pumps and pressure monitors. Photograph of the TFF set-up includes hollow fiber, pumps, processing bag, pressure sensors and monitors (A). Flow rates and pressures are monitored and regulated throughout the process and recorded in real time (B). The TFF process requires ~1-2 hours and incorporates washes and buffer exchange resulting in a ~10-fold volume reduction of the cells in which viable cell concentration is also monitored in real time (C). The T-cells from the TFF process represent >85% of the total input cells with no impact in viability (D) or phenotype (E). The recovered cells remain sterile and can be cryopreserved or stored in an appropriate buffer for long term storage and shipment.

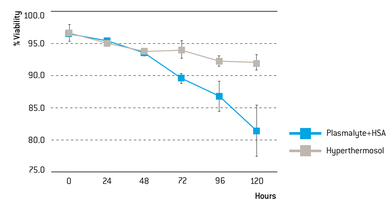


Figure 7. **Long term viability is maintained over time with Hypothermosol at 4°C.** Cultured T-cells may need to be infused without a cryopreservation step, thereby requiring a storage buffer that supports long term viability. T-cells from a WAVE bag culture were harvested and resuspended at 100 × 10⁶ cells/ml in either Hyperthermosol or Plasmalyte/HSA buffers, and stored at 4°C for 5 days. Cell counts and viability were assessed daily and demonstrated that for long term storage (>3 days), Hyperthermosol maintained greater viability than Plasmalyte/HSA. Each time point represents a mean of triplicates +/- standard deviation.

2. Conclusion

Patient-specific cellular therapies hold tremendous potential for being able to treat life threatening diseases where there are currently no cures. In this set of studies we optimize a model T-cell expansion protocol for yield and phenotype, and demonstrate:

- Large scale processes capable of producing billions of T-cells within 10 days among various platforms
- Utilized a single use and fully disposable TFF system for the concentration and clarification of these cells
- Extended the non-frozen shelf-life of T-cells using GMP-compatible formulation buffers.

By applying best practices in process development to patient-specific processes, we have the potential to take products to market that are therapeutically robust, and at reasonable costs to enable effective commercialization.

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