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Application Note

CAR-T Therapy Manufacturing with the ekko™ Acoustic Cell Processing System

Harvest of Expanded T cells

Background

Chimeric Antigen Receptor T cell (CAR-T) therapies have spearheaded a renaissance in immunology and regenerative medicine. As these therapies gain approval, bottlenecks are moving from the clinic to the manufacturing site. Here, manufacturing technologies that provide consistent, closed, and automated solutions to unit operations are required to enable robust and affordable therapeutic production.

Harvest using the ekko™ Acoustic Cell Processing System

Once a therapeutic T cell population has been expanded, it is critical that this material is concentrated and prepared in a formulation buffer prior to taking QC samples and cryopreserving the final product. FloDesign Sonics' ekko™ Acoustic Cell Processing System enables user-friendly automation of wash and concentration steps required for the harvest. The system is a functionally closed, integrated manufacturing platform that gently processes cells while reducing media and removing cell debris from the target cell population.

System Overview

The ekko™ Acoustic Cell Processing System is a GMP capable platform technology for cell and gene therapy manufacturing. The system uses acoustophoresis to hold cells at low energy locations in a three-dimensional standing wave based on their size, density and compressibility. As acoustic technology provides a wide operating window, the system can be optimized for a variety of unit operations throughout cell processing workflows, including concentration and cell washing. Intuitive controls and a purpose-built, single-use cartridge make the system a flexible and scalable tool for early stage research through GMP manufacturing.

Setup

Either a transfer bag or a bioreactor containing the input cell material is connected to the ekko™ Acoustic Cell Processing System via sterile welding of industry standard PVC or C-Flex tubing. Cell harvest requires 2 connections from the transfer bag or the bioreactor containing the input material to the ekko™ Acoustic Cell Processing System. If magnetic particles are used for selection or activation, then debeading can occur before or after concentration within the system. DMSO-compatible materials allow processing of both formulation and cryopreservation buffers within the system.

Figure 1. ekko™ Acoustic Cell Processing System.

T Cell Harvest Protocol Steps

1. **Prime** ekko™ chamber with buffer
2. **Recirculate** cells through ekko™ chamber with acoustics turned ON to seed acoustic waves
3. **Concentrate** cells in ekko™ chamber while flowing excess media to waste
4. **Wash** cells in ekko™ chamber into final formulation buffer
5. **Collect** cells in final product bag at specified volume

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Process Development

The ekko™ Acoustic Cell Processing System provides a flexible platform that can be optimized to increase recovery, decrease processing time, or control output volume from lot-to-lot. Performance is improved by changing the recirculation time, the acoustic power/flow rate ratio, and the settling time. Optimal power/flow rate ratio and recirculation time parameters can be determined by titrating the parameter and measuring the retention of cells within the ekko™ chamber. Similarly, settling time can be optimized via a titration strategy and comparing final products. Performing these studies at both the lower and upper limits of target cell numbers ensures the robustness of protocols developed

Materials & Methods

Two different methods to produce T cell products were used for comparison. In one process, PBMC's were isolated from apheresis products, activated with Dynabeads® (3:1 bead/cell ratio), and cultured for 6 days in shake flasks followed by 2-4 days in a 3 Liter Mobius® bioreactor. Cell counts and viability were determined using a Vi-Cell XR. In a second process, PBMC's were isolated from apheresis products and activated with OKT3. Cells were then expanded for 10-14 days in a G-Rex® platform. Cell phenotype was determined using flow cytometry.

Results

Harvest of expanded T cell products showed consistent recovery among several samples and donors. The ability to develop multiple protocols enabled similar outputs from two heavily different processes. No significant changes in T cell viability nor phenotype were seen due to ekko™ Acoustic Cell Processing System.

Conclusion

The ekko™ Acoustic Cell Processing System is a closed, automated, and consistent platform for processing

T cell products and allows for:

- Tunable parameters to enable process optimization accounting for input cell variability
- Repeatable outputs across multiple donors and cell batches
- High recovery of T cells
- Preservation of T cell phenotype and viability

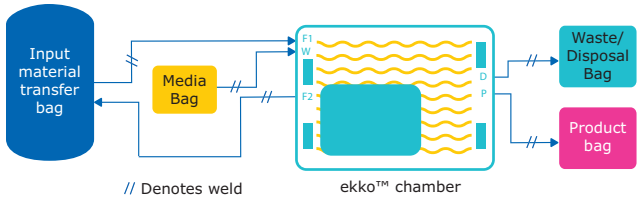

Figure 2. Process Flow Diagram for T cell harvest procedure using ekko™ system.

Process Inputs	ekko™ processing w/ magnetic beads	ekko™ processing w/o magnetic beads
Volume (mL)	823±230	973±49
Total Cells (e9 cells)	1.6±0.3	0.92±0.04
Cell Viability (%)	95.9±0.4%	98.7±0.2%
Process Outputs		
Volume (mL)	82±2	91.4±0.2
Total Cells (e9 cells)	1.4±0.2	0.78±0.05
Cell Viability (%)	95.6±0.8%	95.9±0.9%
Process Performance		
Viable Cell Recovery (%)	89±5%	87±6%
Process Time (minutes)	40.0±5.8	56.4±2.4
Viability Change (%)	-0.3±0.6%	-2.8±1.0%

Table 1. Result of T cell harvests from two different methods. n=6 runs (n=3 donors) performed with Dynabeads® (magnetic). n=4 runs (n=2 donors) performed with OKT3 (nonmagnetic).

Process Inputs	ekko™ processing w/ magnetic beads	ekko™ processing w/o magnetic beads
Volume (mL)	985±27	498±1
Total Cells (e9 cells)	1.6±0.1	1.7±0.6
Cell Viability (%)	96.1±0.3%	95.5±0.4%
Process Outputs		
Volume (mL)	84±2	80±1
Total Cells (e9 cells)	1.4±0.1	1.5±0.4
Cell Viability (%)	95.8±0.7%	95.2±0.8%
Process Performance		
Viable Cell Recovery (%)	88±5%	90±6%
Process Time (minutes)	43.7±3.2	32.7±0.2
Viability Change (%)	-0.3±0.7%	-0.4±0.3%

Table 2. Result of T cell harvests at different volumes & cell numbers. n=4 runs performed with the high-volume process. n=2 runs performed with low-volume process.

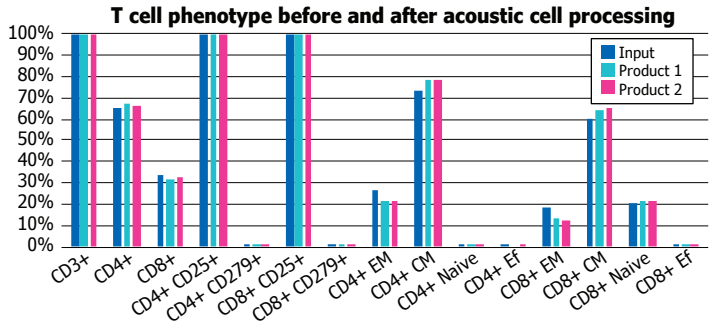

Figure 3. Comparison of T cell surface markers pre & post ekko™ processing. Similar phenotypic breakdown seen in all samples.

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