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Application Note

Integrated Pluripotent Aggregate Processing with the ekko™ System

Background

Pluripotent stem cells (PSCs) and induced pluripotent stem cells (iPSCs) are promising sources for the next wave of cell therapies and regenerative medicine. As with many cell therapies, one major technical hurdle is the scalable production of PSCs for commercial applications, specifically when processing these cell types in aggregate form. Traditional technologies have not been able to deliver a closed and automated solution to gently process PSC aggregates that does not adversely impact cell viability, morphology, function, or efficacy.

Automated Media Exchange using the ekko™ System

Performing media exchanges throughout the expansion and/or differentiation processes of PSC cultures is generally a multi-step open process. Our ekko™ system enables automation of the media exchange and subsequent harvest steps. The system is a functionally closed, integrated manufacturing platform which gently processes cell aggregates and provides the option to remove any free single cells.

ekko™ System Overview

The ekko™ 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 ekko™ can be used and optimized for a variety of unit operations throughout the production workflow, inlcuding but not limited to concentration and/or wash. Intuitive controls and a purpose-built single-use cartridge make the ekko™ system a flexible and scalable tool for early stage research through to GMP production.

Experimental Setup

The system is sterile-welded to the cell source with industry standard PVC and C-Flex tails to enable connection to a variety of bioreactor and transfer bag types.

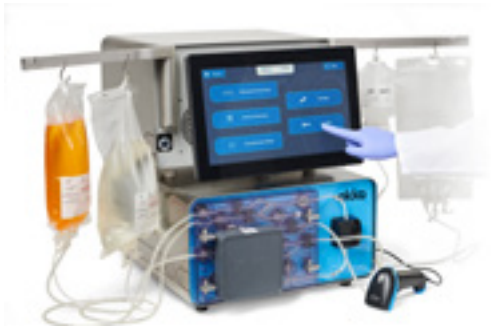

Figure 1. ekko™ Acoustic Cell Processing System.

Aggregate media exchange processes require two (2) connections between submerged ports on the bioreactor and the ekko™ system to allow aggregates to return to the bioreactor. Once the ekko™ system has been attached to the bioreactor it can be used for multiple media exchange steps, as well as bioreactor harvest, ensuring closed loop processing.

Media Exchange Process Steps

1. Prime ekko™ chamber with fresh media
2. Flow input material from bioreactor to waste bag with acoustics ON, capturing aggregates & depleting single cells
3. Return captured aggregates in ekko™ chamber to the bioreactor every X minutes (cell type dependent)
4. Repeat steps 2 & 3 until desired bioreactor volume removed
5. Drain ekko™ chamber back to bioreactor and rinse chamber with fresh media
6. Add fresh media to the bioreactor

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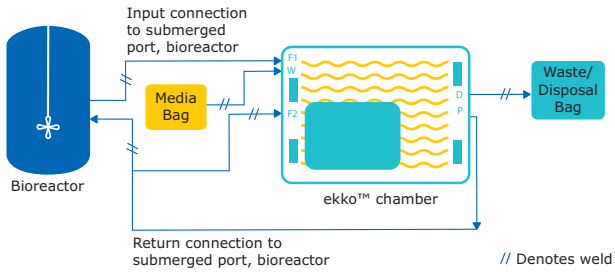


Figure 2. Process flow diagram for aggregate media exchange process using ekko™ system

Process Development Method

Depending on the composition of the bioreactor and the objectives of the media exchange process, the ekko™ system can be tuned to either preferentially deplete single cells or retain cell aggregates. This tuning is based on the ratio of acoustic power to flow rate and can be determined empirically by performing a power titration experiment and measuring the waste stream. Since ekko™ system performance varies based on aggregate and single cell size and type, measuring the retention is recommended to determine the optimal settings for best performance for your cell line.

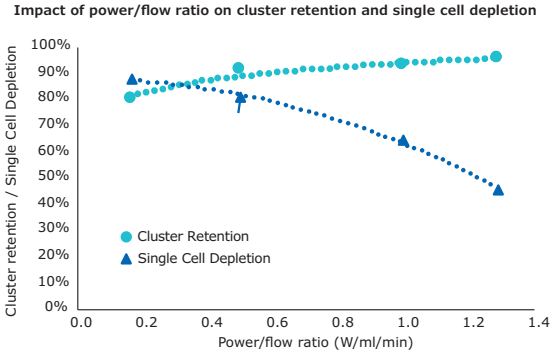


Figure 3: Output power titration curve on PSC aggregates with a size range of 100-200 µm demonstrating impact of power/flow ratio on cluster retention (blue) and single cell depletion (orange).

Results

Media exchanges performed on PSC cultures with different power/flow ratios demonstrated similar trends to the process development outcomes. Microscopy of the input and acoustically processed material show no significant differences in morphology. Following the media exchange process PSC were kept in the reactor for an additional three (3) days with no observed issues.

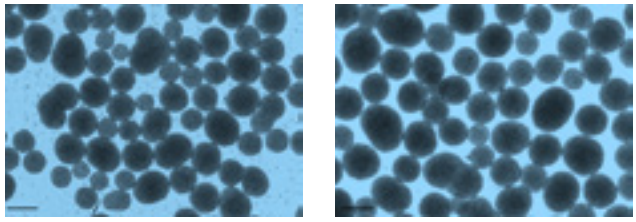
Conclusion

The ekko™ Acoustic Cell Processing System is a closed, automated, and gentle platform for processing PSC cell aggregates and allows for:

- efficient media exchanges with minimal loss of aggregates across multiple exchanges
- preferential and highly tunable removal of single cells providing retention of aggregates for return to the bioreactor
- high recovery of aggregates harvested post expansion, with no impact to morphology cell function or differentiation potential

Table 1. Result of aggregate media exchange processes at power/flow ratios of 0.5 and 1.0. Improved aggregate recovery and reduced single cell depletion is seen with increasing ratios. Data was obtained using the ViCell instrument (Beckman Coulter). Aggregate cells were dissociated using Accutase®.

	Experimental value	
Process Inputs	**P/F Ratio 1.0**	**P/F Ratio 0.5**
Volume (mL)	2100	2000
Total Single Cells (e9 cells)	2.5	2.6
Viable Clustered Cells (e9 cells)	1.2	0.9
Clustered Cell Viability (%)	92.3	95.2
Process Outputs		
Volume (mL)	2200	932
Total Single Cells (e9 cells)	1.6	0.2
Viable Clustered Cells (e9 cells)	1.2	0.8
Process Performance		
Viable Clustered Cell Recovery	>99%	91%
Single Cell Depletion	37%	93%
Process Time (80% media exchange) (minutes)	39.2	41.1
Viability Change, Clustered Cells (%)	-1.3	-3.9



Pre-process **Post-ekko™**

Figure 4. Agg Media Exchange App note cell images Representative microscopy images of PSCs, pre and post ekko™ processing. The scale bar represents 200 µm.

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