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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. For many of these cell therapies, a key to the scalable production of PSCs for clinical and eventual commercial applications is expansion and differentiation in a 3D format; specifically, processing these cells in aggregate form. Traditional technologies have not been able to deliver a closed and automated solution to gently process PSC aggregates that do not adversely impact cell viability, morphology, function, and/or efficacy.

Automated Media Exchange using the ekko™ Cell Processing System

Performing media exchanges throughout the expansion and differentiation processes of PSC cultures is generally a multi-step open process. Our ekko™ Cell Processing System enables automation of the media exchange and subsequent harvest steps. The system is a 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 uses acoustophoresis to trap and hold cells at locations of low acoustic pressure amplitude (nodes) in a sound field based on their size, density, and compressibility. As acoustic technology provides a wide operating window, the ekko™ System can be used and optimized for a variety of unit operations throughout the production workflow, including but not limited to concentration and/or wash. Intuitive controls and a compact single-use assembly designed for easy error-proof setup makes the ekko™ System a flexible and scalable tool.

Figure 1. ekko™ Acoustic Cell Processing System.

Experimental Setup

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

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

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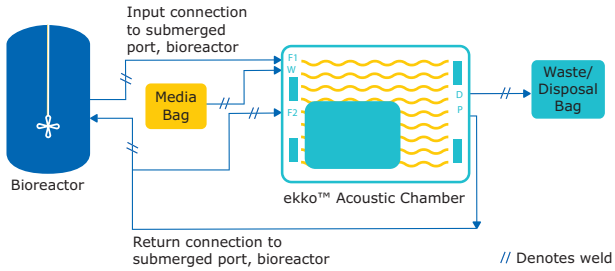


Figure 2. Process flow diagram for aggregate media exchange process using ekko™ System

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, we recommend measuring the retention during process development to determine the optimal settings for best performance for your cell line.

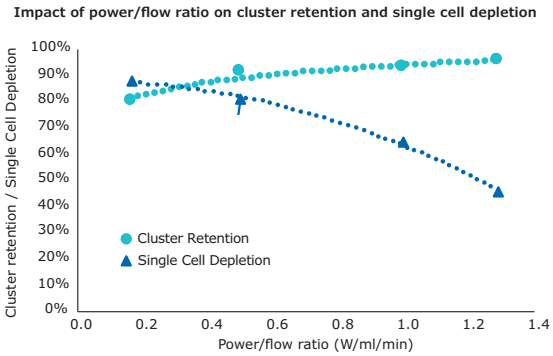


Figure 3: Output power titration curve on PSC aggregates with a size range of 100-200 µm demonstrating impact of power/flow ratio on cluster retention (cyan) and single cell depletion (blue).

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 was kept in the reactor for an additional three (3) days with no observed issues.

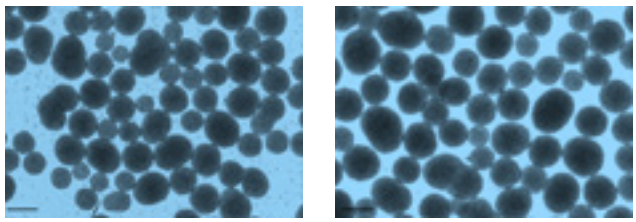
Conclusion

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 and cell viability

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 VI-CELL® 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™ System**

Figure 4. Representative microscopy cell images of PSCs, pre and post ekko™ System processing. The scale bar represents 200 µm.

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Results are experimental and were obtained using a prototype ekko™ Single Use Assembly, which may be representative of the final product.