Flow Cytometry Research Group 2014 Study

Evaluating Effects of Cell Sorting on Cellular Integrity and Gene Expression

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Introduction

During the past year the Flow Cytometry research Group has continued on its goal to establish best practice guidelines for cell sorting conditions that minimize cell stress, perturbation, or injury to the sorted cells.

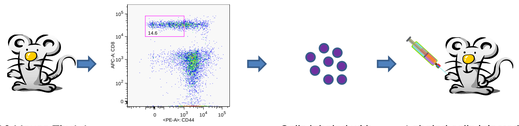
Towards this goal the group has followed up on an observation from our initial study that showed poor cell recovery when a clonal population of cells (Jurkat) was sorted aggressively under intentionally adverse sorting conditions (excessive pressure as well as undersized sorting orifice). In this follow-up study we sought to identify unique qualities of the cells that survived the adverse sorting conditions, in the hope that this may prove to be a useful test method for assessing deleterious effects of cell sorting across a wide variety of cell types.

To address this question, six FCRG member-sites received a distribution of the same Jurkat cell population and using different instrumentation and sorting conditions, sorted these cells for subsequent cell cycle analysis, post-sort viability, and recovered cell counts. In addition, one site submitted parallel samples for microarray analysis.

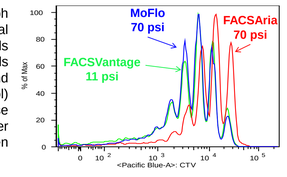
Background

Previous studies by individuals in the FCRG have revealed detrimental effects on cell function after sorting using cuvette versus jet-in-air sorters.

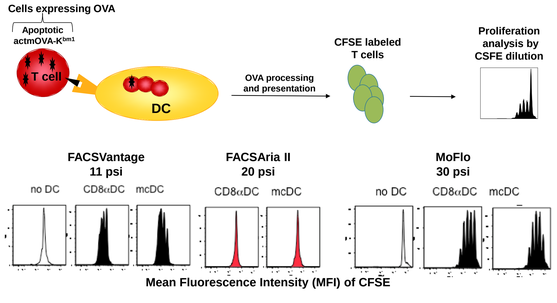
Study 1-Decreased proliferation of transplanted T cells



Mice were sacrificed after 1 week and lymph node and spleen were evaluated for total number of CellTracker Violet labeled cells and the degree of proliferation. The cells sorted on the jet-in-air MoFlo and FACSVantage (low pressure control) proliferated to a greater degree than those sorted on the FACSAria cuvette sorter indicating an increase in cell injury when sorting using a cuvette.



Study 2-Decreased function of dendritic cells



Dendritic cells sorted on the jet-in-air FACSVantage and MoFlo were able to process and present antigen to CFSE labeled T cells resulting in proliferation while those sorted on the FACSAria cuvette sorter displayed a decrease in antigen presenting function.

Jurkat Cell Study

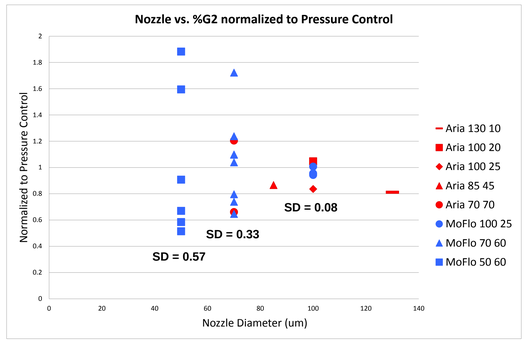
Jurkat cells were evaluated after cell sorting by analyzing cell cycle profile and gene expression changes.

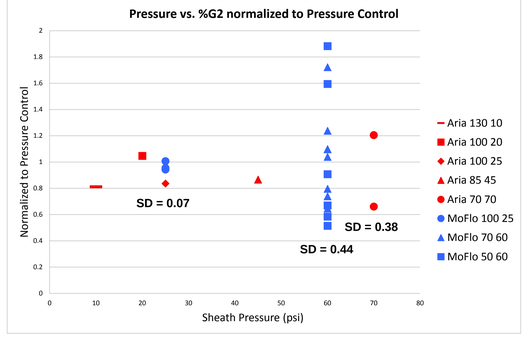
Sample treatments included:

- **Unsorted Control -** Cells that were kept on ice for the duration of the sort
- **Pressure Control** - Cells that were mounted on the sorter and exposed to pressure, but not sorted
- **Sorted Sample** - Cells that were collected after sorting

Cell Cycle Analysis

Preliminary evidence revealed a loss of cells in G2 phase of the cell cycle after sorting under harsh conditions. To determine if cell cycle profile changes are an indicator of adverse sorting conditions, Jurkat cells were distributed to several sites and sorted using a variety of instruments and settings. Control cells were exposed to pressure but not sorted. Viability data was obtained before and after sorting. Cells were ethanol fixed, shipped to a participating site, stained with propidium iodide and analyzed for cell cycle profile. Data points are grouped based on the instrument, nozzle size and sheath pressure respectively (see legend).





The frequency of cells in G2 from the sorted sample was normalized to that of the pressure control. When comparing this normalized value to the diameter of the nozzle tip or to the sheath pressure there is a larger variation using a smaller nozzle diameter and/or a higher pressure.

Gene Expression Data

Flow sorting is often upstream of functional or gene expression studies. We wanted to understand the degree, if any, to which flow sorting may induce changes in gene expression and minimize these effects when possible through use of optimal conditions. Jurkat cells, a robust transformed cell line, were sorted on a MoFlo cell sorter using a 50 um nozzle tip at 60 psi, pelleted and resuspended in culture media and incubated for the times indicated. Gene expression changes were determined using Affymetrix Primeview microarrays and data was analyzed using the TAC software.

Sorted sample vs. unsorted control at 4 and 8 hours

4 Hours

Fold Change (linear)	Gene Symbol	Description
(+)2.04	KMT2C	lysine (K)-specific methyltransferase 2C
-2.04	ACTG2	actin, gamma 2, smooth muscle, enteric
-2.04	TMPO	thymopoietin
-2.1	NNT	nicotinamide nucleotide transhydrogenase
-2.14	HTR2B	5-hydroxytryptamine (serotonin) receptor 2B, G protein-coupled
-2.15	SERPINH1	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)
-2.21	FKBP4	FK506 binding protein 4, 59kDa
-2.22	RRN3 , RRN3P1 , RRN3P2	RRN3 RNA polymerase I transcription factor homolog
-2.27	STEAP1	six transmembrane epithelial antigen of the prostate 1
-2.27	RGS18	regulator of G-protein signaling 18
-2.41	ACTA2	actin, alpha 2, smooth muscle, aorta
-2.45	HSPA4L	heat shock 70kDa protein 4-like
-2.52	CCNB1IP1	cyclin B1 interacting protein 1, E3 ubiquitin protein ligase
-2.83	SCG2	secretogranin II
-3.01	H1F0	H1 histone family, member 0
-5.26	HSPA6 , HSPA7	heat shock protein 6 ,protein 7
-19.9	HSPA1A , HSPA1B	heat shock protein 1A ; 1B

8 Hours

Fold Change (linear)	Gene Symbol	Description
-2.01	ACTA2	actin, alpha 2, smooth muscle, aorta
-2.17	ANKRD37	ankyrin repeat domain 37
-2.19	ACTG2	actin, gamma 2, smooth muscle, enteric
-2.25	VEGFA	vascular endothelial growth factor A
-2.38	PTPN3	protein tyrosine phosphatase, non-receptor type 3
-2.54	VEGFA	vascular endothelial growth factor A
-2.86	DDIT4	DNA-damage-inducible transcript 4
-3.31	VEGFA	vascular endothelial growth factor A

Sorted sample vs. pressure control at 4 and 8 hours

4 Hours

Fold Change (linear)	Gene Symbol	Description
(+)2.23	QSER1	glutamine and serine rich 1
(+)2.06	SBK1	SH3-binding domain kinase 1
(+)2.05	SCRIB	scribbled planar cell polarity protein
(+)2.04	TXLNG2P	taxilin gamma 2, pseudogene
(+)2.03	SFT2D3 , WDR33	SFT2 domain containing 3 ; WD repeat domain 33
(+)2.01	SPEN	spen homolog, transcriptional regulator (Drosophila)
-2.01	SGK494 , SPAG5	uncharacterized serine/threonine-protein kinase SgK494 ; sperm associated antigen 5
-2.05	HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
-2.06	NNT	nicotinamide nucleotide transhydrogenase
-2.16	SLC16A1	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)
-2.19	PRC1	protein regulator of cytokinesis 1
-2.31	BTBD1	BTB (POZ) domain containing 1

8 Hours

Fold Change (linear)	Gene Symbol	Description
No changes	NA	NA

Pressure control vs. unsorted control at 4 and 8 hours

4 Hours

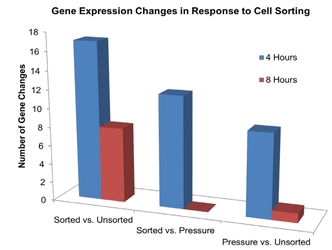
Fold Change (linear)	Gene Symbol	Description
-2.03	CHORDC1	cysteine and histidine-rich domain (CHORD) containing 1
-2.05	HSPA4L	heat shock 70kDa protein 4-like
-2.07	JUN	jun proto-oncogene
-2.14	H1F0	H1 histone family, member 0
-2.16	RRN3 , RRN3P1 , RRN3P2	RRN3 RNA polymerase I transcription factor homolog
-2.41	CCNB1IP1	cyclin B1 interacting protein 1, E3 ubiquitin protein ligase
-2.59	SERPINH1	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)
-6.54	HSPA6 , HSPA7	heat shock 70kDa protein 6 (HSP70B') ; heat shock 70kDa protein 7 (HSP70B)
-16.5	HSPA1A , HSPA1B	heat shock 70kDa protein 1A ; heat shock 70kDa protein 1B

8 Hours

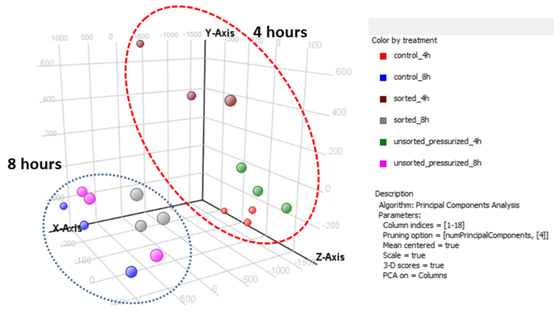
Fold Change (linear)	Gene Symbol	Description
-2.05	VEGFA	vascular endothelial growth factor A

Gene Expression Data

Gene expression changes in Jurkat cells that were sorted and re-cultured for 4 hours are minimal and decrease substantially after 8 hours of culture. Indicating a minimal effect caused by the sorting process and that Jurkat cells can recover upon exposure to normal culture conditions.



Principle Component Plot of Microarray Data



A principle components analysis of the microarray data suggests that the sorting introduces some cellular changes at the transcriptional level but these changes substantially decrease after a recovery period.

Conclusions

- Functional data from certain cell types reveals detrimental effects from cell sorting using a cuvette style instrument.
- Cell cycle profile changes are highly variable in Jurkat cells sorted using smaller nozzles and/or higher pressures.
- The highest number of up-regulated genes was detected 4 hours after sorting when comparing sorted cells to those exposed to pressure only (sorted sample vs pressure control) indicating an immediate gene expression response from the sort.
- There is a minor effect of pressurizing the cells which causes only down regulation of genes (pressure control vs. unsorted)
- From these data it appears that most changes come from the actual sorting process. However, it is important to note that the overall effect of sorting is surprisingly minimal and that Jurkat cells can recover after a resting period.

Acknowledgements

We sincerely appreciate the support of Affymetrix Inc. for Primeview microarrays, reagents, and data analysis. Stowers Institute Cytometry and Tissue Core Facility for providing the Jurkat cells. Marcy Kuentzel for microarrays analysis at the Center for Functional Genomics at SUNY Albany.