Large Particle Flow Cytometry Allows High Quality Isolation of Viable Cardiomyocytes and Other Myocytes

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Introduction

Isolated cardiomyocytes (CMs) and other myocytes are valuable models to study disease mechanisms of various pathological conditions. Genomic and physiological changes lead to changes in contractility and other cellular dysfunctions. Although CMs numerically represent a minority of heart cells, they represent most of the heart mass. Isolating CMs is a challenge and current biochemical analyses of heart proteins do not correlate the content of CMs-specific proteins with the number of cells in normally developed tissue (e.g. blood vessels) or with heart function [1].

Myocytes are rod-shaped fragile cells and their isolation from tissue requires special techniques, buffers and culture conditions. Single-cell analysis research with these delicate cells is difficult and limited. Sorting of CMs using conventional FACS instruments results in frequent clogging and a high percentage of dead cells. For example, RNA-seq data from single-cell isolation using a Fluidigm C1 platform shows the prevalence of mitochondrial reads indicating cell damage [2].

Here we show examples of successful mouse CMs and skeletal myocytes isolation using large particle flow cytometry, LP-FACS (COPAS™ and BioSorter®). These instruments allow isolating large and fragile cells without damage. Myocytes isolated using COPAS and BioSorter maintained their contractile function and generated RNA-seq libraries more representative of intact muscle cells. Overall, this approach enables high-throughput purification of CMs and skeletal muscle cells for cellular assays, functional analysis and sequencing.

Methodology

For these studies, COPAS and BioSorter platforms were used. These instruments automate the analysis, sorting and dispensing of large and fragile cells as well and cell clusters (10µm-1500µm). It provides a flexibility for the sizes of object that can be analyzed, generating measurements for size parameters (TOF, Time Of Flight), optical density (EXT, Extinction), internal complexity (FSC, forward scatter; SSC, side scatter) and the intensity of fluorescence markers. Once analyzed, objects are sorted according to user-selectable criteria and then may be dispensed into stationary bulk receptacles or multi-well microtiter plates. To avoid damaging or changing the fragile samples, a gentle pneumatic device located after the flow cell is used for sorting and therefore makes the instrument suitable for handling live biological materials. (Fig. 1). The fluid pressures of the instrument (up to 6 psi) are significantly lower than those of traditional flow cytometers, generating lower shear forces and less damage to the sample objects.

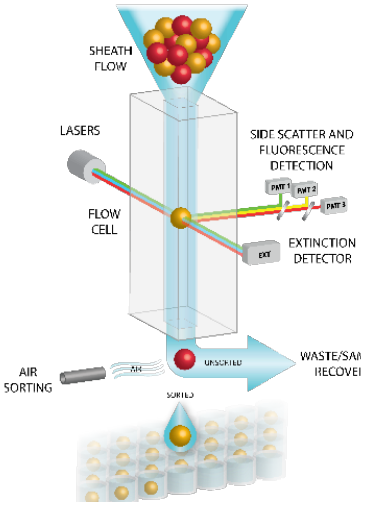

Figure 1. Analysis and sorting of objects inside the flow cell. Objects are carried through the flow cell by a liquid stream while their physical properties are being measured. The convergence of the sheath and sample fluid allows "hydrodynamic focusing" of the objects, channeling them to go through the center of the flow cell along their longitudinal axes.

Results

(A) -- Cardiomyocytes isolation and purification

Here we discuss the results of isolation of viable mouse CMs and skeletal myocytes using COPAS large particle flow cytometry. Cardiomyocytes were isolated from adult (>3 month of age) C57l/6J mice. Cells were prepared using Langendorff dissociation method through heart perfusion. Cells were analyzed and sorted using Sony SH800S cell sorter (100 and 130 um chips) and Union Biometrica COPAS with 500um flow cell at Johns Hopkins University [3]. Upon sorting cells were analyzed for shape (Figure 2) and scRNA-seq library preparation (Figure 3).

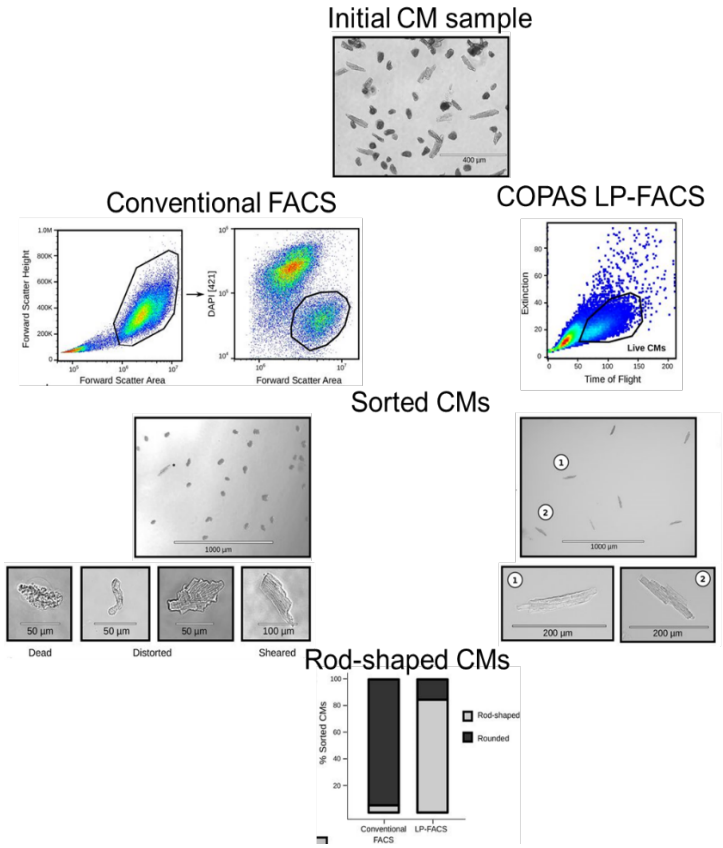

Figure 2. Purification of mouse cardiomyocytes (CMs) using different flow cytometry methods. Mouse hearts were dissociated using Langendorff perfusion methods. CMs were sorted using Sony SH800S and Union Biometrica COPAS 500. ~200-400 cells were analyzed per sorting approach. Adapted from https://doi.org/10.1101/654954

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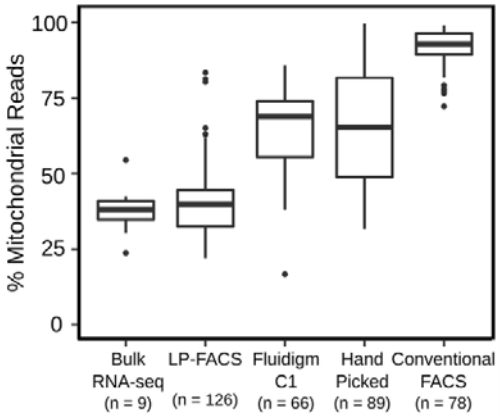
The quality of isolated cardiomyocytes was assessed by analyzing transcriptomics data. A high percentage of mitochondrial reads in the data suggest cell membrane damage leading to cytosolic RNA leaking out of the cells. CMs isolated with COPAS had a lower number of mitochondrial reads compare to conventional FACS (Figure 3). Thus, large particle flow cytometry can be used to generate high-quality CMs for functional and transcriptomics experiments.



Method	Median number of mitochondrial reads, %
Bulk - not purified	38.15
COPAS - LP-FACS	**39.88**
Fluidigm C1 -Microfluidics	68.91
Manual - hand picking	65.23
Sony - Conventional FACS	92.97

Figure 3. Genomic analysis of mouse cardiomyocytes isolated using COPAS flow cytometer. Percentage of mitochondrial reads in transcriptomic data from isolated cardiomyocytes using different methods. Median values are listed in the table. Adapted from https://doi.org/10.1101/654954.

(B) -- Mouse skeletal muscle fibers isolation

Mammalian skeletal muscle tissue consists of different types of muscle fibers. They have different physiological properties which lead to the functional diversity of muscles. Such diversities have been related to differences in the amount and isoforms of contractile proteins and signaling receptors. For example, fast muscle fibers are primarily affected in Duchene muscular dystrophy [4]. Single-cell functional analysis of myocytes has been challenging due to their fragility. Furthermore, muscular dystrophies make skeletal muscles even more difficult to isolate. Skeletal muscle fiber cells may become damaged during contraction in Duchene patients.

Here we report successful isolation of live mouse skeletal muscle fiber cells (myocytes). The experiments were performed using a BioSorter (Union Biometrica) with a 500µm flow cell at George Rodney lab, Baylor College of Medicine, Houston, TX.

Using a label-free gating approach we were able to purify live mouse skeletal muscle fibers. Live sorted fibers continued to contract after sorting providing a cell isolation method for further physiological and biochemical analysis.

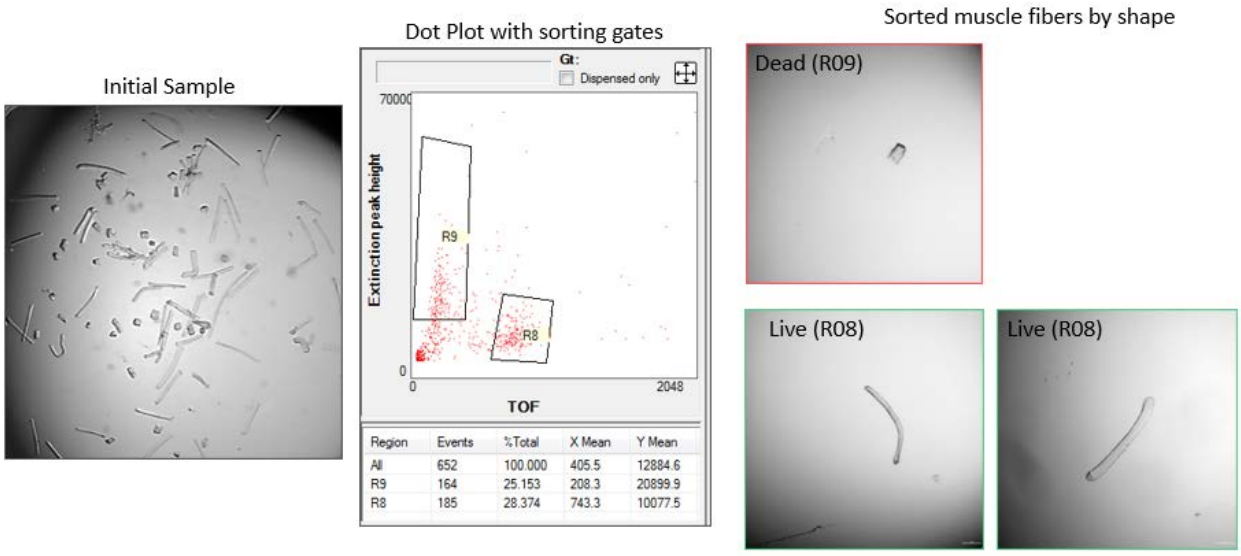


Figure 4. Purification of mouse skeletal muscle fibers. Mouse skeletal myocytes were sorted using Union Biometrica BioSorter with 500 µm flow cell. Contractions of live sorted fibers was verified by inspection using a stereo microscope.

Conclusions

These experiments demonstrated that BioSorter and COPAS LP-FACS instruments can be used to isolate both cardiac and skeletal myocytes. We demonstrated that sorting is fast, reproducible and does not damage these large delicate cells. The automated analysis and sorting process is gentle and does not affect morphology or viability. The quality of post-sorted cardiomyocytes was validated by RNA-Seq analysis and skeletal myocytes by retention of contractility.

The BioSorter and COPAS LP-FACS provide a level of automation to the process of handling and isolating myocytes allowing for increased throughput and elimination of any biases that might be introduced by the researcher. Single cell isolation is a tedious task and the increased throughput provides opportunities for large scale studies such as drug discovery assays and toxicology studies in multi-well formats. Such increased throughput can be valuable for study of muscle-cell diseases and the use of cardiomyocytes in adverse drug effect toxicology studies. The data is collected and analyzed electronically and made available for easy incorporation into presentations and reports.

The BioSorter and COPAS LP-FACS instruments bring the methods of flow cytometry to the analysis and sorting of cardiac and skeletal myocytes which are otherwise too large and fragile for analysis on conventional single-cell flow cytometers. These instruments bring the advantages of flow cytometry, specifically statistically meaningful data, large unbiased data sets, and multiparametric analysis, to experiments using myocytes.

Acknowledgments

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References

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