Method for Analysis and Sorting of Live Adipocytes

Work performed May 2012 by:
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Objective

Increasing rates of obesity and diabetes in the human population indicate the need for further research into the underlying biology, including the biology of adipocyte tissue. Such studies could benefit from advancements in the tools available for analysis and isolation of cells, with modifications to accommodate adipocytes. Many questions about adipocytes remain that require separation of adipocytes from the other cells that populate adipocyte tissue, such as inflammatory cells. Up to now no adipocyte specific surface marker has been identified. We have developed a method for high-throughput analysis and sorting of intact adipocytes based on the nuclear stain (Hoechst). Sorted cells were intact and could be used for downstream applications including imaging and sequencing assays.

Introduction

Purified adipocytes present a valuable tool for studying physiological processes in diabetes, obesity and other metabolic disorders. Fluorescence activated cell sorting (FACS) is a common tool in cell isolation and purification. Traditional FACS has been used previously for sorting of embryonic stem cell (ESC) derived adipocytes (Schaedlich K., et al., 2010). However traditional FACS instruments are capable of sorting only a limited size range of cells and will miss the population of larger cells (>70um). It has been shown that adipocyte size and fatty acid composition is changing during weight loss (Verhoef S.P. et al. 2013). It is quite likely that the biology differs for the different sized cells. Unique physiological properties of adipocytes present additional challenges for traditional fluorescence activated cell sorting. Here we describe a method for analyzing and sorting of primary mouse adipocytes using a BioSorter® large particle flow cytometer (Fig. 1). Cells of similar size were sorted together, separating the broad size range of adipocytes into collections of cells of similar size. These cells remained intact after sorting. We believe this capability of analyzing and sorting intact adipocytes is not broadly available with most other flow cytometers.

Figure 1. The BioSorter is a multi-range analyzer and sorter that allows high-throughput handling of objects from 10μm to 1500μm.

Preparation of Adipocytes

Primary mouse adipocytes were freshly prepared from epididymal fat pads. Fat tissue was removed, minced, and digested using collagenase (0.625 mg/ml) at 37°C for 40 min. The primary adipose cells were then washed extensively in a Dulbecco's Modified Eagle Medium (DMEM) w/o Phenol Red containing 2% of bovine serum albumin (BSA) and centrifuged for 5 min at 900rpm. Primary adipose cells were then stained with a nuclear dye (Hoechst 34580 392/440 nm) and Calcein (495/515nm) live/dead stain. The cell suspension was then loaded into the sample cartridge and analyzed/sorted using a BioSorter® large particle flow cytometer. The BioSorter instrument was equipped with a 500μm metal-free FOCA (Fluidics and Optics Core Assembly). DMEM or PBS (pH=7.4) with 2% BSA was used as a sheath solution during experiments. Physical characteristics of size (TOF), optical density (EXT), and three different channels of fluorescence signals (FLU) were collected. Sample objects were carried through the flow cell, one by one, and passed through the focus of a laser beam. Relative size was determined by the time of flight (TOF) measurement. The optical density of the object was determined by the extinction (EXT) measurement. Using Profiler II™ we collected spatial information on fluorescence and extinction for each object passing through the flow cell. Solid state 405nm and 488nm lasers were used in the experiments. Calcein fluorescence was collected using BP510/23 nm filter (PMT2) and Hoechst 34580 fluorescence using BP 440/30 nm (PMT1).

Results

Primary mouse adipocytes are an important physiological model to study metabolic disorders. We have developed a special sample introduction system and tested the ability of BioSorter® to analyze and sort intact adipocytes. This proprietary flow-through rotating sample chamber gently disperses, without damage, fragile samples that otherwise float (like adipocytes) or samples that sink. Two different size populations were prepared using a 40µm cell strainer. Cells were analyzed and sorted using a 500µm FOCA (designed for objects 20 to 400µm) on the BioSorter instrument (Fig. 1). Live adipocytes were selected based on Calcein (515nm) stain and presence of the nucleus (Hoechst, 440nm). Using FlowPilot™ software we were able to gate the population of adipocytes (Fig. 2A). Profiler II allowed identifying single cell population (Fig. 2B).

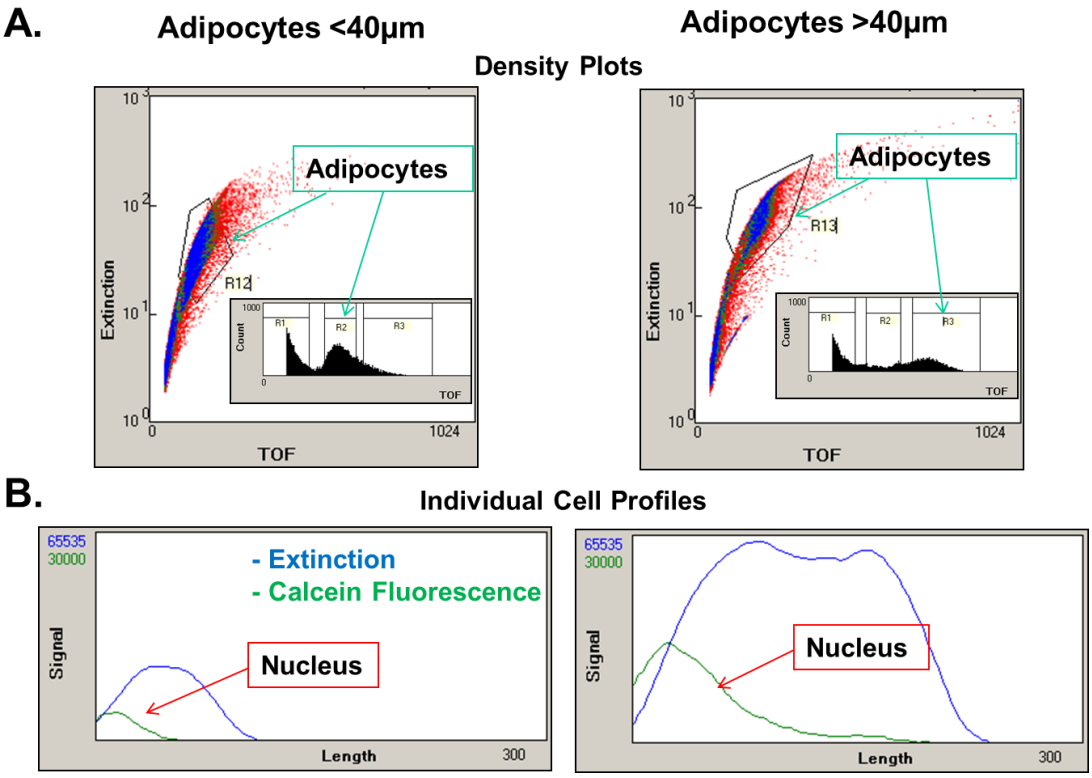

Figure 2. Flow cytometry analysis of primary mouse adipocytes. A. Selecting population of cells based on size and extinction. B. Representative profiles of single cells in selected gates. Extinction was recorded from 488nm laser. Calcein fluorescence was recorded at 515nm. Data was analyzed and plots generated in FlowPilot software.

We also performed successful gentle dispensing of adipocytes. Cells were sorted directly onto cover slips and imaged using Zeiss Axioscope microscope with 20x

objective (Fig. 3). We were able to achieve sorting with greater than 70% of adipocytes intact in the post-sort population.

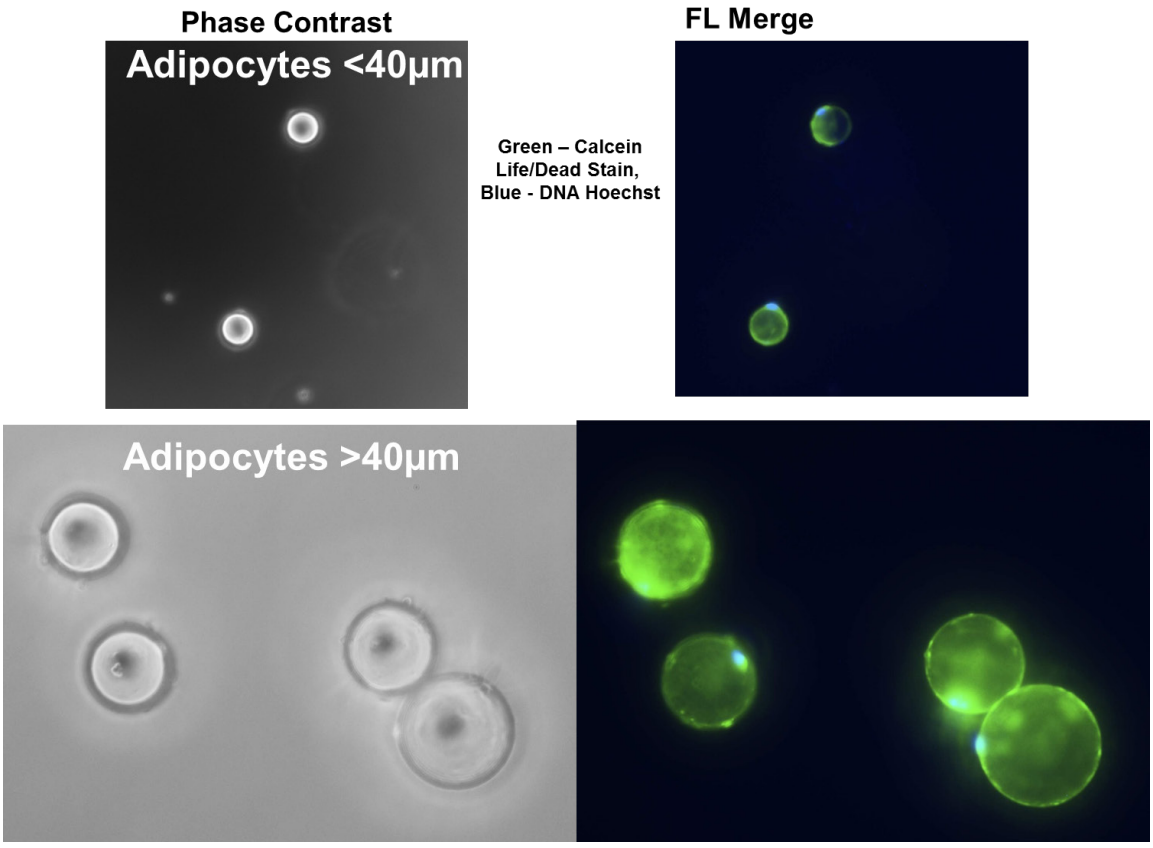

Figure 3. Sorting of primary mouse adipocytes using BioSorter. Adipocytes were sorted to slides and counted under the microscope. At least 100 cells were counted for each sort.

Conclusions

We developed a method and tool for the high speed gentle sorting of fragile cells, specifically primary adipocytes. This approach can be used both for analysis and sorting of these cells. The method allowed sorting of freshly prepared adipocytes and more than 70% of these cells remained intact following dispensing. We determined that the larger microfluidics channel of the 500 FOCA was better for collecting intact adipocytes. This is likely due to the lower pressures and the reduced stream velocity resulting in lower shear forces.

Acknowledgments

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