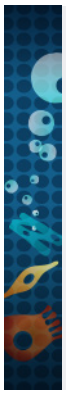

Cell Sorting and Its Significance in Stem Cell Research

By Gunjan Choudhary

Despite the fact that stem cells were discovered more than three decades ago, cell sorting has, until recently, been limited to large institutional core facilities. Now, stem cell researchers can start taking advantage of the power of benchtop cell sorters and their ability to improve results.

Stem cells possess the remarkable potential to self-renew and to differentiate into many different types of cells. In fact, it is these two characteristics that distinguish stem cells from other cell types. Due to their unique ability to regenerate, stem cells have been used in the regeneration of tissues and organs over the past two decades, albeit with varying degrees of success (Bolli et al. 2011, Schwartz et al. 2012). Stem cells also offer great hope for treating diseases such as diabetes and heart disease. Given their importance in regenerative medicine and in normal physiology, stem cells are very extensively studied. To that end, there has been an increasing need to develop techniques that allow the separation and isolation of highly pure populations of stem cells in a manner that is efficient, simple, flexible, and easy to use.

Cell sorting is one extensively used technique to isolate a pure stem cell population from a heterogeneous suspension of cells and is a powerful and highly precise and sensitive method with high resolution (Zhu and Murthy 2013). Cell sorting allows the separation of cells based on their intra- or extracellular properties, including DNA, RNA, and protein interactions, size, and surface protein expression. Specifically, a heterogeneous population of cells is passed, in a steady stream of single cells, through an electronic detection apparatus, which, in conjunction with a laser beam, analyzes the cells based on their physical and chemical characteristics and tells the instrument to either sort the cell or send it to waste. Though cell sorting used to be regarded as a complex and costly method that required special training, this has changed with the advent of versatile, user-friendly, personalized benchtop cell sorters. They provide stem cell researchers with the ability to easily and quickly learn to sort stem cells in a gentle yet powerful and efficient manner.

In one specific type of cell sorting, known as fluorescence-activated cell sorting, antibodies tagged with fluorescent dyes are mixed with cells, to which they attach. These tagged cells are then individually sorted based on their fluorescence and light scattering properties. This method results in cell populations that are >95% pure (Zhu and Murthy 2013). One of the first fluorescence-activated cell sorting methods used to isolate populations of stem cells was side population analysis, which was based on the ability of stem cells to efflux Hoechst 33342, a DNA labeling dye, at a rate faster than that of more mature cells. This is a unique attribute of many stem cell populations, including hematopoietic, embryonic, and cancer stem cells. However, though side population analysis can isolate stem cells from non–self-renewing cells, it cannot distinguish between the different types of stem cells, which leads us to the second type of fluorescence-activated cell sorting.

In this method, populations of stem cells are isolated based on the proteins expressed on their cell surface and within the cell. Since each type of cell expresses a unique set of molecules, or markers, they can easily be distinguished using the appropriate fluorescent antibodies. Antibodies used in cell sorting can be conjugated to fluorophores, such as fluorescein isothiocyanate (FITC), phycoerythrin (PE), and allophycocyanin (APC) among others, or fluorescent proteins, such as GFP, RFP, and mCherry among others. Thus, fluorescence-activated cell sorting is an integral part of stem cell research, allowing for both the isolation and characterization of stem cell populations.

Since it was introduced in the mid to late 1960s, fluorescence-activated cell sorting has been primarily incorporated into the fields of human clinical medicine and biomedical research, specifically in immunology, pathology, oncology, and molecular

biology (Preffer and Dombkowski 2009). High-quality cell sorting instruments with multiple lasers have been available since 2002. At that time, they required a highly trained individual to use them.

Before their inception, cell separation techniques needed high-power UV ion-laser sources with costly infrastructural support, which prevented side population analysis and the ability to perform UV or near violet excitation assays (Cabana et al. 2006, Kapoor et al. 2007). However, with the advent of these high-quality cell sorters, high-efficiency optics, electronics, and fluidics became possible, and these features worked together to greatly improve the resolution of cell sorting instruments and reduce their costs. These advances have also eliminated the need for specialized training to use the cell sorters and allowed cell sorters to have greater design adaptability and, hence, smaller footprints (Preffer and Dombkowski 2009).

One such cell sorter is Bio-Rad's S3e Cell Sorter, which measures forward scatter (FSC), side scatter (SC), and several fluorescent parameters and is capable of both high-speed analysis and sorting. The S3e Cell Sorter has recently been used successfully to sort various types of stem cells, which have, in turn, been used in a variety of downstream applications. In one such study, the S3e Cell Sorter was used to sort pure populations of mesenchymal stem cells from various mouse tissues, which were used to carry out proteomic analysis and elucidate key signaling pathways in cell therapy applications (Sudheer and Bose 2017). In another study, the cell sorter was used to isolate human primordial germ cell-like cells (hPGCLCs) from competent human pluripotent stem cells (hPSCs) to better understand the causes of infertility and germ cell tumors (Irie and Surani 2017). Other applications of the cell sorter include the development of a human lung from lung bud organoids (LBOs), which were differentiated from purified hPSCs (Chen et al. 2017) and the elucidation of the regulation of the euchromatic state of purified embryonic stem (ES) cells (Bulut-Karslioglu et al. 2015).

With intuitive, specialized software and easy-to-use automation, the S3e Cell Sorter is the ideal partner for stem cell researchers looking to sort highly pure populations of stem cells for in vitro and in vivo characterization and other downstream molecular analyses.

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