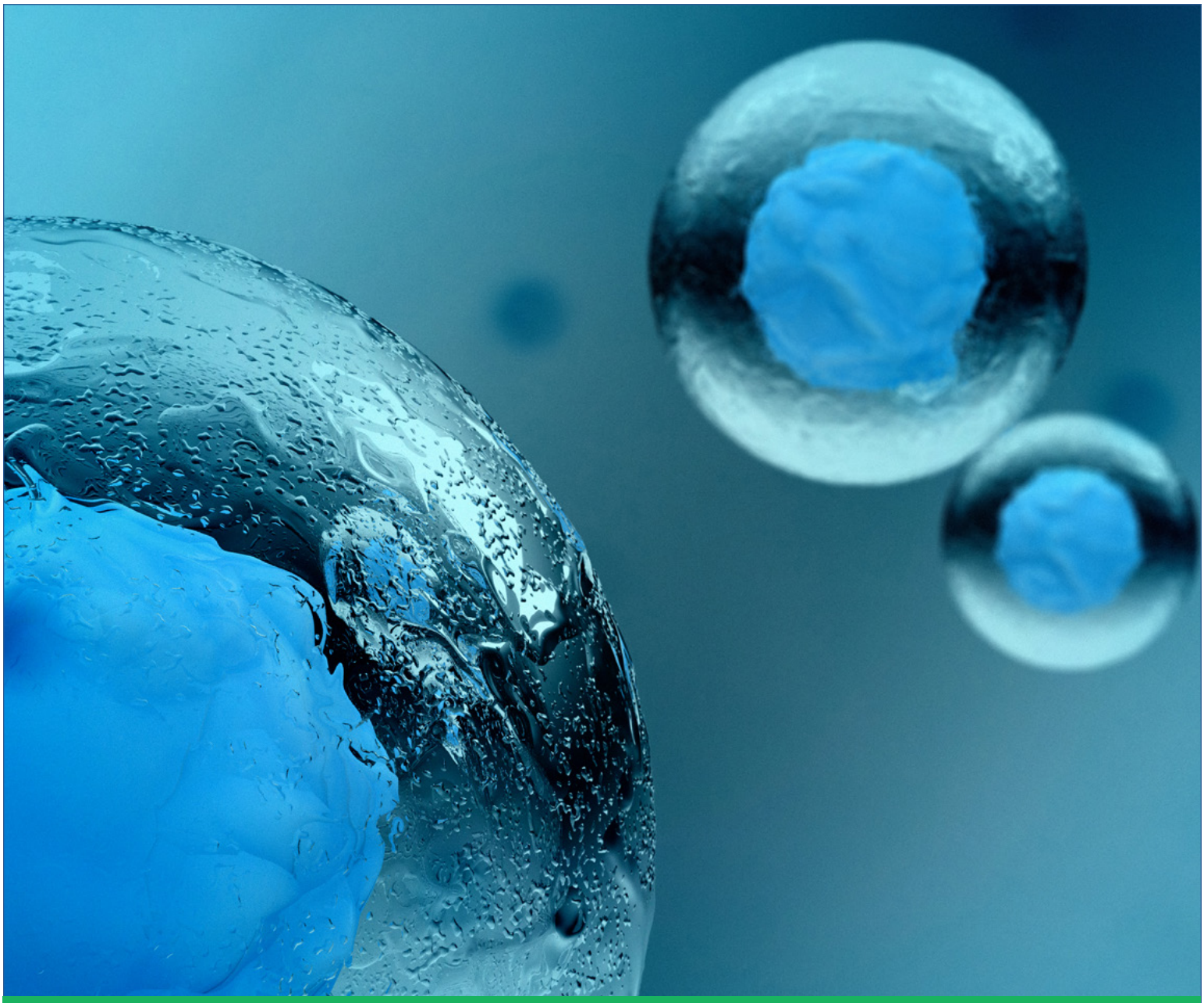

Using Fluorescence Activated Cell Sorting In Preclinical and Clinical Research

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Many researchers across biomedical disciplines face a situation when they need to isolate a pure population of cells. They might be stem cells for basic research or a patient's T cells that can be engineered to attack their own tumor. The many uses for pure cell populations in both the preclinical and clinical research has created a demand for techniques that preserve the viability and functionality of cells and optimize the purity of the cell population of interest. Fluorescence activated cell sorting (FACS) is a flow cytometry-based technique that is the workhorse of biomedical research because it can satisfy these criteria. This white paper highlights the many applications of FACS in both basic and clinical research, and how cell sorting techniques can be customized for the specific downstream uses of the sorted cells.

Hardware

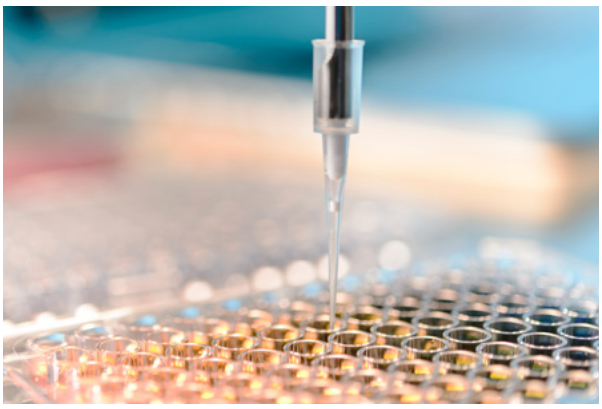
Cell sorters work much like flow cytometers. A stream of fluorescently labeled cells passes through a laser and the laser excites the fluorescent molecules. Lasers are defined by the wavelength of light emitted, and different fluorescent molecules, such as FITC, APC or Pacific Blue, have specific excitation wavelengths that cause emission of photons at a different wavelength, which is measured by photodetectors and used to sort cells based on fluorescent labeling parameters. Sorters can house multiple lasers and photodetectors, so multiple unique cell populations can be sorted during a single run. Sorters also contain closed fluidics systems that operate under different pressure parameters, which can be critical if high-throughput applications are needed.

As a highly sophisticated piece of lab equipment, a cell sorter is also prone to technical challenges. The nozzle that takes up the cell suspension into the sorter can clog easily, especially if sample is not properly prepared as a single cell suspension. Lasers can go out of alignment and the fluidics system can suffer from wear and tear. Cell sorters require vigilant daily maintenance and regular service to assure that they function as expected. Many cell sorters are maintained in core facilities and handled by experience cell sorting technicians, but any researcher can learn the basics of cell sorting and cell sorter maintenance.

Sample Sources

The quality of the starting sample is critical to sorting success. Samples may come from diverse sources such as peripheral blood, bone marrow, lymph nodes or cell culture. Samples must be single cell suspension and handled to assure maximum cell viability, and special steps are typically included at the end of sample staining, like running samples through cell strainers and using special sorting buffers, to maintain single cell suspensions. Staining protocols should also include viability dyes so dead cells can be discriminated from live cells as most researchers prefer working with live cells as a sorting end product. Some staining protocols call for using a cell fixative like paraformaldehyde to fix cells after staining, but fixation steps can promote cell clumping so additional rinsing steps are recommended with fixed samples.

Sample Concentration



The sample concentration and volume dictates critical sorting parameters, especially the speed of sorting. Highly concentrated samples can clog the nozzle or other parts of the fluidics system, but highly dilute samples may result in a sorted sample with few viable cells due to a longer sort timing. It is critical to determine what particular sorter you will use, as machine-specific parameters will determine what cell concentrations can be run at low or high sorting speeds. This information will also help you determine in what volume the starting samples should be suspended so you can achieve the correct cell concentration.

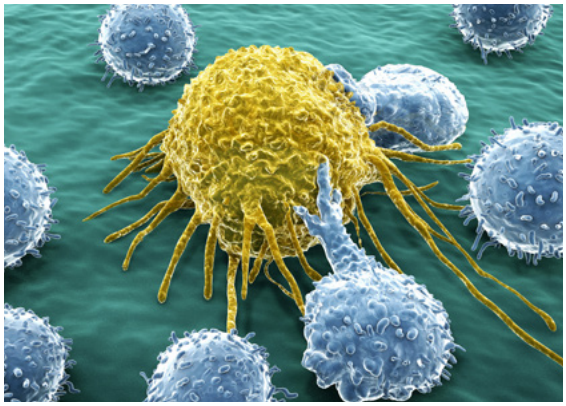
Although overall concentrations are critical, an understanding of the relative rarity or abundance your sorted population is also critical to calculating your sorting parameters. Certain cell subsets, such as specific T cell subsets, may be relatively abundant in your starting specimen, so you can achieve your final sorted sample concentration relatively easily. In contrast, sorting rare cell populations, like certain dendritic cell subsets, will require a large input sample in order to sort enough cells to work with downstream.

Many cell sorting protocols have been optimized with an enrichment step prior to FACS. Magnetic-activated cell sorting (MACS) techniques can complement FACS protocols by enriching samples with particular cell types prior to sorting. MACS involves incubating cells with immunomagnetic beads coated with specific antibodies, and cells bound to magnetic beads are separated when a magnet is applied to the sample. MACS can be used for positive selection in which your cells of interest are bound to beads and can be recovered from them. In contrast, negative selection means that cells you are excluding from selection are bound to beads and your cells of interest are enriched in the unbound fraction of sample. MACS is a convenient and flexible option for cell enrichment prior to FACS and can be done by researchers prior to sending samples to core facilities or CROs for sorting.

How Clean? Aseptic or Non-Aseptic Sorting

Consider your downstream applications when deciding under which conditions you need sorted cells to be handled. Cell sorters are typically managed as aseptic or non-aseptic machines. Aseptic sorting is intended for samples that will be used in animal models or patients or need to be maintained aseptically for experimental reasons. Non-aseptic sorting is sufficient for cells being analyzed in vitro for different immunological properties and is suitable for many basic research applications. Stringent maintenance and compliance requirements are typically required to maintain aseptic sorters and may require that your samples meet certain aseptic criteria.

Downstream Applications

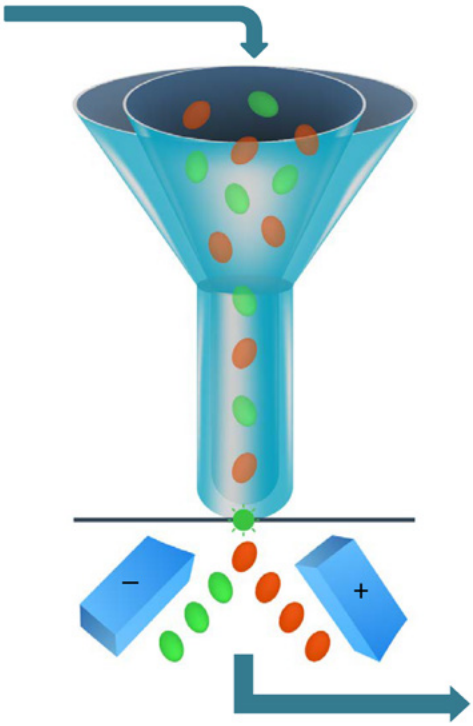


Cell sorting is a critical part of many basic immunology experiments that involve characterizing the phenotype and function of immune cell subsets, and how interactions between cells trigger critical immune responses. Sorting is especially helpful for studying rare cells, and numerous protocols have been developed to optimize sorting of such cells[1,2]. FACS has been essential to current advances in single-cell sequencing technology, and single cell genome, transcriptome and epigenome studies are providing unprecedented insights into how the immune system works and is influenced by somatic mutations[3]. Single cell sequencing has become a valuable tool for cancer research in particular and has facilitated both the foundational understanding of how different cancers work as well as informing novel drug development[4].

Beyond the lab bench, cell sorting has been instrumental to clinical advances in transplantation and immunotherapy. Specialized sorting methods have been developed to prepare clinical grade cell products for use in humans that meet good manufacturing practice (GMP) criteria and other regulatory requirements . Cell sorting will continue to be a critical tool in this era of precision medicine when a patient's cells can be isolated and re-engineered to fight cancer.

Final Considerations

Cell sorting is a powerful and flexible tool that is used widely for both basic and clinical research. Many cell sorters are managed by skilled staff scientists and technicians, and researchers benefit by working together with sorting experts to design and execute experiments. If large sorting projects are at hand, working with a contract research organization is an option that may be better suited to handle a larger scale project. Consider the many ways sorting can enhance your research projects.



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