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FLOW CYTOMETERS AND THEIR APPLICATIONS IN CLINICAL RESEARCH

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ABSTRACT

Flow cytometry has quickly risen to the status of a standard analytical tool in medical research due to its robustness. Flow cytometry is a powerful tool that may examine individual cells within a larger population. Similar to microscopy, this method automatically quantifies certain optical properties of the cell or cell population under study using a flow cytometer. Flow cytometry can be used to learn about cell size, number of cells, chromosomes, and biological processes including apoptosis and cell adhesion. When it comes to diagnosing diseases, flow cytometry is important for the following tasks: a complete count of blood cells Cell sorting has many applications in biology, including the study of various leukocyte types, the identification of pathogenic microbes in environmental and biological samples, the determination of total DNA content in cells during tumor biopsies for cancer research, the sorting of T cells to assess the impact of infections on their function, and the detection of minimal residual disease cells in bodily fluids.

KEYWORDS: Flow Cytometer, Blood cell, B cell, T Cell, Leukemia, DNA content.

INTRODUCTION

One tool for studying cellular processes, such as light scattering and fluorescence emission, is flow cytometry, which uses an optical-to-electronic coupling mechanism. Injecting a population of cells or particles suspended in a fluid into a flow cytometer allows for the measurement and detection of their physical and chemical properties (1).

A flow cytometer is useful for measuring many characteristics of a suspended particle. The flow cytometer works by injecting a sample, which may contain cells or other materials—into a fluid that suspends them. A steady stream of particles in suspension is illuminated by a single-wavelength light beam. It is common practice to label cells with fluorescent markers that absorb light and then emit it within a specific wavelength range (1-2). As they float through the air, the suspended particles disperse the light, which detectors positioned perpendicular to the beam capture. Emitted light from these excited complexes of fluorescent compounds coupled with tiny particles has a lower frequency than that of the light source (3). Detectors then collect this light and evaluate it based on the variations in detector brightness or fluorescence. Transmitting light and creating real-time photographs of each fluorescent cell is the final product. The three parts that make up flow cytometry are optics, electronics, and fluidics (1,4). In a fluidics chamber, the optics comprise a light source and detectors, while the electronics include a computer for signal analysis, a system for amplifying linear or logarithmic signals, and a digital-analog converter that produces fluorescent size-complexity parameters and signals. Monoparametric histograms, dot plots, and contour plots are all possible ways to store the data collected from a flow cytometer. In a dot plot, the number of cells is plotted against the fluorescence intensity, and in a contour plot, the closer the rings are to each other, the denser the concentric ring population (2).

All of the top biomedical research organizations employ flow cytometry for activities that demand great throughput and analytical precision. All throughout the globe, you may find them in medical centers and hospitals as well. Ploidy, cell cycle, and cancer surface analysis are the main diagnostic uses. Surface indicators of lymphoma and leukemia are also studied using them. Additionally, it has been utilized for assessing CD4 lymphocyte counts in blood, which helps track the course of AIDS and the response of patients to treatment. Immunodeficiency condition evaluation, hematological anomalies, tumor cell analysis in circulation, and intracellular molecular detection are some of the major clinical uses (5-6).

Flow cytometry equipment has come a long way in the

previous few decades. Common examples of purpose-built equipment include systems with several lasers, systems that integrate microscopy and flow cytometry, systems that integrate mass spectrometry and flow cytometry, and systems with 96-well loaders designed for bead analysis.

Enhanced This unit will provide a general outline of the various instrumentation platforms that are now available. The number of fluorochromes, including tandem dyes and polymer dyes, utilized to conjugate monoclonal antibodies has increased dramatically (1,3). Furthermore, a wider variety of fluorescent proteins, like as mCherry, mBanana, mOrange, mNeptune, etc., have been available for transfection, expanding the options beyond GFP. Because of these developments in fluorochromes and instruments, studies with the potential for 30+ parameters have been conducted. So in this review we summarized some important diagnosis which has been done by flow cytometer and play a very important role in diseases diagnosis and treatment (1,7-8).

What flow cytometry can be used for

Phenotypic characterization of cells

Analyzing blood cell phenotypes Immunophenotyping, also known as phenotypic characterization of cells, involves the use of flow cytometry to identify and quantify a specific cell type within a mixed population. One example of this is the immune cells found in blood. Antibodies allow for the detection of certain markers, which are proteins found on the surface of cell membranes. More than one kind of cell can express these particular antigens. The vast majority of immune cells are uniquely identified by a set of CD markers. Each immunophenotyping investigation makes use of these cell markers, which are known as lineage markers, to identify distinct cell populations for further research. This includes NK cell markers (CD56, Cd161), monocyte markers (CD14, CD11b), T cell markers (CD3, CD4, CD8), B cell markers (CD19, CD20), and so on (9-10).

Antigen binding response

Quantifying antigen-specific responses can be accomplished in a number of ways, one of which is by using MHC multimers to detect inflammatory cytokine, proliferation, t-cell and b cell activation, and recognition. By stimulating cells with a particular antigen, another method can be utilized (11). It is common practice to biotinylate the MHC monomers (I or II) connected to a fluorescent streptavidin backbone. The number of MHC monomers that make up a multimer can range from four (tetramer) to ten (dextramer). These MHC multimers attach to T cells that identify the antigen after being "loaded" with the desired antigen; this shows how strongly the immune system reacts to that antigen. In vaccine research, this application is used rather often (12).

Apoptosis detection

Flow cytometry allows for the quick and accurate detection of apoptotic cells. If we want accurate findings from our methods for detecting cells going through apoptosis (programmed cell death), we need to make sure that the cells can maintain their original state while we quickly identify their apoptotic characteristics. Various flow cytometric techniques have been detailed for the evaluation of cell death, and DNA fragmentation (13-14).

Viability of cell

Various types of cells rely on cell viability measurement. Results on the percentage of live cells versus dead cells can be obtained from a viability test. False positives and incorrect conclusions could come from non-specific antibody binding caused by non-viable cells in your samples. Flow cytometry depends on a simple but powerful premise to evaluate cell viability. Flow cytometry makes it easy to determine cell viability by adding a DNA-binding dye to a cell population at a relatively low concentration. Several fluorochromes, can be used in flow cytometric analysis to differentiate between dying and living cells (8,12,15).

Identification of membrane potential

There are a variety of physiological and morphological changes that distinguish apoptosis from other cell death processes. The alteration of the cell's membrane is one of the first steps that occurs in an cell death process. The integrity of the plasma membrane can't be compromised in any way during the process of the membrane phosphatidylserine translocation, which takes place from the inner leaflet to the leaves on the outside. This translocation is responsible for the detection of death since it leads to the loss of plasma membrane integrity, which is the outcome of the process (14,16).

Mitochondrial proteins identification

The endoplasmic reticulum, nuclear envelope, and mitochondrial outer membranes all contain the human proto-oncogene Bcl2. Evidence from preclinical research shows that Bcl2 acts as an intracellular anti-apoptotic protein, and a decline in Bcl2 levels is associated with an increase in cell death. Fixation and permeabilization of cells were necessary steps in detecting Bcl2 activity. The next step was to use flow cytometry to examine the cells after adding a fluorescence-conjugated Bcl2 antibody (17-18).

Cytokine Analysis

Two protein transport inhibitors are administered to cells for 2-12 hours to facilitate intracellular cytokine analysis. This allows for improved detection of cytokines within the cell. Vaccine peptides and other antigens can be introduced to cells during incubation to gauge the immune response. After cells have been stained for viability markers and cell surface markers, they are fixed and permeabilized for cellular markers using anti-cytokine antibodies, and treatment with protein transport inhibitors (7,19-20).

Different Stages of Cell, Cycle Analysis

A DNA-binding dye saturating solution is used to stain DNA in cell cycle analysis tests. Staining with a dye (PI, 7AAD, DAPI) follows typically fixing the cells in a 70% ethanol solution, which permeabilizes the cells. Nevertheless, there are dyes like Hoescht 33342 that can stain DNA without harming living cells. Analyzed utilizing ploidy modeling software, this method determines the cell cycle stages by acquiring samples at a low flow rate using linear amplification (21-22).

New advances in the field of flow cytometry

As a result of the discovery of quantum dots (Qds), flow cytometry technology has made significant progress. This is because quantum dots have opened up new opportunities for highly multiplexed tests that have improved resolution. Quantum dots made of semiconductors like selenium and cadmium stand out from the previously mentioned tiny and tandem dyes because they are unique fluorescent nanocrystals with dimensions ranging from 2 to 6 nanometers and special characteristics. As a result of their broad absorption spectra, quantum dots (QDs) are able to be stimulated by a wide range of wavelengths, in contrast to FITC and PE, which both require an absorption spectrum. Furthermore, at wavelengths greater than 300 nm, they exhibit a high degree of photostability, and a single source is capable of simultaneously exciting all of their colors. When it comes to detecting cells and other particles at a high resolution, modern spectral flow cytometers perform better than their more traditional equivalents in terms of sensitivity and speed. Spectral flow cytometers, in contrast to conventional flow cytometers, make use of innovative optics and detectors, which enables them to obtain outstanding results. Spectral flow cytometers make use of prisms and gratings as dispersive optics, whereas typical flow cytometers make use of optics (dichroic mirrors) to either transmit, block, or reflect a photon depending on the wavelength of the photon. Quantifying the continuous spectrum that is produced by the prism or grating is normally accomplished through the utilization of a detector array in a linear fashion in the process of spectral flow cytometry (23-24). Presently, multi-junction photomultiplier tubes (PMTs) and charge coupled devices are utilized as detectors in spectral flow cytometers. The majority of the time, spectral flow cytometry has been utilized for validation investigations, and efforts are currently being made to investigate its biological applications. As an illustration, a recent study made use of spectral flow cytometry to investigate five different fluorescent proteins created for bacteria in isolation from one another. This was done to ensure that there was no spectrum overlap.

CONCLUSION

In recent years, the introduction of new chromophores and various types of flow machines has significantly contributed to the fields of cancer diagnostics and immune phenotyping. Flow cytometry is a highly effective approach for analyzing many types of material quickly. This strategy yields substantial information about the issue of interest. Although there have been notable improvements in flow cytometry throughout the past century, the organization has consistently introduced new ideas to reduce expenses, dimensions, and intricacy, while concurrently enhancing sensitivity. More efficient fluorochromes, such quantum dots (Qds), and sophisticated flow cytometers, like spectral and microfluidic flow cytometers, are among the innovations discussed in the article. Efforts to improve and expand flow cytometry will continue, resulting in notable progress in various fields like basic science, medicine, and biology.

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