FACS

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FACS (Fluorescence Activated Cell Sorting)

Introduction

The FACS (Fluorescence Activated Cell Sorting) is a laboratory technique that allows to examine AND SORT millions of cells, both normal and tumoral cells, in a very short time and receive a lot of information on their biological behavior. It is a specialized type of flow cytometry, a technique that permits EXAMINATION AND SORTING of cells suspended in a fluid medium. The effectiveness of this particular technique is the ability to measure multiple properties of individual cells at a very fast rate, allowing a detailed qualitative and quantitative analysis. It allows to obtain different information about structures and functions of individual cells through the analysis of some physical parameters (diffraction, refraction, reflection, fluorescence) that characterize a beam of light after it has interacted with every single cell of the test sample.

Technique

The process begins by placing the cells into a flask and letting the cells to enter a small nozzle one at a time (figure 1). The cells travel down the nozzle which is vibrated at an optimal frequency to produce drops at fixed distance from the nozzle. The system is adjusted so that there is a low probability of more than one cell per droplet. As the cells flow down the stream of liquid, they are scanned by a laser. Some of the laser light is scattered by the cells and this is used to count the cells. This scattered light can also be used to measure the size of the cells. If you wanted to separate a subpopulation of cells, you could do so by tagging those of interest with an antibody linked to a fluorescent dye. The antibody is bound to a protein that is uniquely expressed in the cells you want to separate. The laser light excites the dye which emits a color of light that is detected by the photomultiplier tube, or light detector. By collecting the information from the light (scatter and fluorescence) a computer can determine which cells are to be separated and collected. The final step is sorting the cells, which is accomplished by electrical charge. The computer determines how the cells will be sorted before the drop forms at the end of the stream. As the drop forms, an electrical charge is applied to the stream and the newly formed drop will form with a charge. This charged drop is then deflected left or right by charged electrodes and into waiting sample tubes. Drops that contain no cells are sent into the waste tube. The end result is three tubes with pure subpopulations of cells. The number of cells is each tube is known and the level of fluorescence is also recorded for each cell.

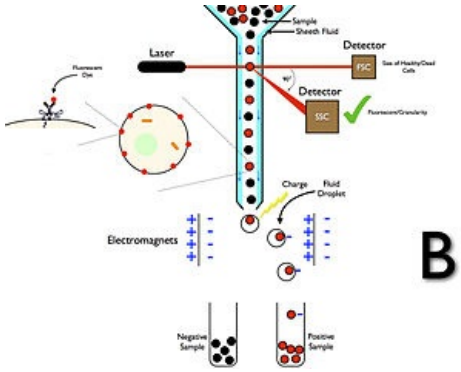

Figure 1, Diagram of FACS machine

Relevance in medicine

It is extensively used in research for the detection of DNA damage. One of the first applications of FACS in fact was the analysis of the position in the cell cycle, performed by quantification of cellular DNA. This is still one of the major technique in tumor detection. In cancer cells is often seen a change in DNA content, the main consequence of chromosomal and subcromosomal genetic changes, having a key role in the development and course of the disease. In the simplest method, the content of DNA is detected using a fluorescent dye able to bind DNA with high affinity. Another of the major applications of FACS is represented by the analysis (and sorting) of the various populations of blood cells, having an important role in immunophenotyping. Over all in medicine FACS has a broad application varying from the methods explained above to transplantation and prenatal diagnosis.

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