Analytica Chimica Acta 965 (2017) 9–35

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Review

Continuous flow microfluidic separation and processing of rare cells and bioparticles found in blood – A review

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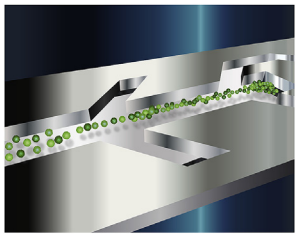
HIGHLIGHTS

- Microfluidic rare cell separation reviewed.
- Needs and advantages for rare cell separation.
- Conventional methods for rare cell separation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form
31 January 2017
Accepted 3 February 2017
Available online 20 February 2017

Keywords:
Microfluidics
Rare cell
Cell separation
CTC
nRBC

ABSTRACT

Rare cells in blood, such as circulating tumor cells or fetal cells in the maternal circulation, posses a great prognostic or diagnostic value, or for the development of personalized medicine, where the study of rare cells could provide information to more specifically targeted treatments. When conventional cell separation methods, such as flow cytometry or magnetic activated cell sorting, have fallen short other methods are desperately sought for. Microfluidics have been extensively used towards isolating and processing rare cells as it offers possibilities not present in the conventional systems. Furthermore, microfluidic methods offer new possibilities for cell separation as they often rely on non-traditional biomarkers and intrinsic cell properties. This offers the possibility to isolate cell populations that would otherwise not be targeted using conventional methods. Here, we provide an extensive review of the latest advances in continuous flow microfluidic rare cell separation and processing with each cell's specific characteristics and separation challenges as a point of view.

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http://dx.doi.org/10.1016/j.aca.2017.02.017
0003-2670/

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1. Introduction

An early encounter with rare cells was done by pathologist Thomas Ashworth, in 1869, when he noticed some unusual cells in the blood of a deceased cancer patient. The cells he had found did not look like normal blood cells but where instead similar to the cells found in the numerous cancer tumors found in the patient's body. Ashworth speculated that these cells could be derived from the tumors and would explain the amount of tumors found in the patient [1]. Since then the cancer cells found in blood, known as circulating tumor cells (CTC), have been proven to be derived from the primary cancer tumor but science has not yet come to an agreement of whether all of these cells or just subset of them have the potential to form new secondary tumors. The great interest in CTCs is due to the fact that they are recognized for their diagnostic and prognostic value, as are many other rare cell populations.

The advent of the Lab-on-a-Chip and Micro Total Analysis System (μTAS) concept in the early 1990s, where the aim is to shrink an entire laboratory with all its functions onto a microchip, eventually presented new tools for the rare cell research area. Compared to conventional methods for cell separation, that are often associated with higher sample loss volumes and lower recoveries, microfluidics has also introduced the opportunities to separate cells based on non-traditional biomarkers and intrinsic cell properties.

Rare cells are commonly several orders of magnitude lower in abundance compared to their background cell population and are often found in an abundance of less than 1000 cells per mL of biofluid.

1.1. Requirements and advantages for rare cell isolation

Rare cells in blood are recognized for their high diagnostic and prognostic values. However, as they reside in a high background population there is a need to separate them from this volume in order to detect or analyse them. The volume of a standard clinical blood sample is around 5 mL, comprising around 5 billion RBCs, 300 million platelets, and 5–10 million WBCs, per mL, in which a rare cell population typically can be found in numbers of 1–1000 cells. Since it is not feasible to draw several hundred mL of blood from a patient some needs and requirements must be fulfilled in order to successfully isolate the rare cells.

First of all, since the cells are low in numbers and the sample volume to be processed is limited, the recovery of them must be high. The recovery of a real sample can of course not be measured as it can never be known if all target cells are collected and detected. Recovery data is, therefore, commonly generated with spiked cell lines. Alternatively, a relative recovery can be generated by benchmarking the separation method against a Golden standard method.

A high recovery will minimize the volume of sample that will need to be processed in order to collect a sufficient number of cells to enable analysis and thus also the processing time of the sample. A high recovery will also ensure that the cells can be correctly enumerated when this is needed. It is not possible to specify a general recovery level needed, but the needed level is dependent on the subsequent analysis and will in turn determine how informative this analysis can be. Furthermore, a high recovery will also ensure that the collected cells are representative of the whole cell population and that the isolation method is not biased in any way.

It is worth noting that comparing recovery data between different experiments can sometimes be misleading. Imagine a simple system composed of a piece of tubing connected to two syringes, pumping sample from one syringe to the other. The recovery of target cells in the collecting syringe is in this example 100%. A high recovery can thus be obtained without actively manipulating the sample at all, *i.e.* the obtained purity is equally low as in the original sample. While some methods will produce 0% recovery when only pumping the samples through the system without actively manipulating them, other methods will in the same way recover some percentage of sample (with the same composition as the input sample) although without sorting it. This will obviously increase the chances for the latter method to generate higher recovery levels also when actively manipulating the samples. This will, however, be at the expense of the purity.