Miniaturized device for a cell lysis process

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Single-cell studies are crucial for gaining knowledge on complexity of intracellular processes. In many cases, carrying researches into cell ingredients must be proceeded by a lysis process. Cell lysis leads to disintegration of the plasma membrane which is the barrier separating cell contents from the environment. However, investigations at the cellular level would not be possible without proper miniaturized tools, which offer many advantages as low reagents consumption, short reaction time, integration, automation or versatility.

The goal of this work was to design and develop a microfluidic chip for a chemical cell lysis process. The geometry of a microsystem presented is based on the hydrodynamic focusing of a cell suspension stream. Applying non-denaturing cell lysis buffer enables to analyze released cell ingredients during next steps of investigations.

Keywords and phrases: microdevice, microfludic chip, chemical cell lysis, sheath flow, hydrodynamic focusing.

Introduction

Cells are the basic structural and functional units of living organisms. All basic physiological functions of multicellular organisms are in the cell [1]. Hence, cell analysis attracts increasing interests in biological, medical, and chemical fields. It is essential for understanding organism metabolism and its functions. Determination of such intracellular components as proteins, DNA or RNA is a key element in early laboratory diagnostics of many diseases, in proteomics, genomics or in forensic medicine [2]. Wide knowledge in these fields contributes to development of new diagnostic procedures, or drugs discovery. However, without appropriate equipment and tools, cell analysis would not be possible. Here is a place for miniaturization, which offers many advantages as low reagents consumption, saving space, short reaction time, integration, automation or versatility [3]. Microfabricated microfluidic analytical devices, integrating multiple sample handling processes with the actual measurement step, are referred as *"Lab-on-a-chip"* devices [4].

Cell lysis

Cell lysis is a method or process leading to disintegration of the plasma membrane (see Fig. 1) which is the barrier separating cell contents from the environment [1]. As a result, intracellular molecules are released to the environment. There are many cell lysis methods: thermal, acoustic, mechanical, electrical and chemical. Each of them has strengths and weaknesses, so careful selection of the proper technique is essential for gathering accurate data from single cells.

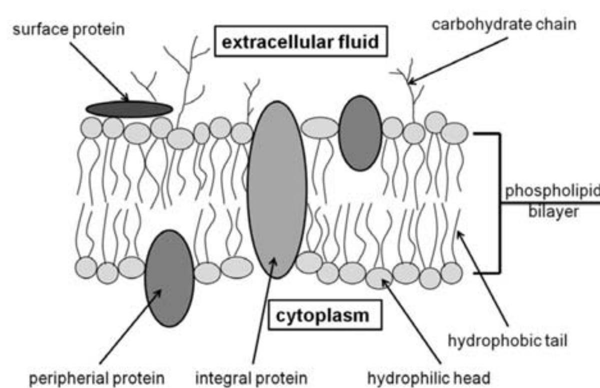

Fig. 1. A scheme of an Eukaryotic cell membrane.

On-chip cell lysis

Different methods of performing cell lysis process, i.e. thermal, electrical, mechanical, and chemical were

successfully applied in microsystems [5]. Depending on downstream applications, certain methods are preferable to others.

Thermal cell lysis is commonly used as a preparatory step in nucleic acids analysis based on PCR (*polymerase chain reaction*). In 1998, Waters et al. [6] reported that thermal lysis of the bacteria could be integrated into the PCR cycle. They described a microchip suitable for complete analysis of multiple DNA samples. However, thermal lysis may cause protein denaturation, so this method is not as readily used as other ones.

Electrical cell lysis is based on electroporation process which causes formation of small pores in the cell membrane and makes it permeable to external medium. It is very popular method due to its speed and reagentless procedure. An exemplary application of electrical lysis was demonstrated by Han et al. [7]. They used gold capillaries and metal-coated glass slides as electrodes to create a voltage field and performed in less than 33 ms lysis of adherent rat cells.

Mechanical methods of cell lysis are also relatively effective and reagentless. Most of them take advantage of shear stress phenomenon to disrupt cells. Di Carlo et al. modified the surface of a microsystem with nanoscale barbs [8]. However, it is difficult to apply mechanical lysis for a single cell analysis.

Chemical methods, which disrupt cell membranes using detergents, solvents and antibiotics, are also widely used for lysis [5]. It is highly economical method of lysis — no expensive equipment such as high-voltage power supplies or pulsed lasers are required. However, it is necessary to take into account the properties of the buffer disrupting the cell. On the one hand lysis buffers may have positive consequences such as the solubilization of membrane proteins and reduction of proteins aggregation, whereas on the other hand they may have undesired effect such as denaturation of proteins. Two most popular detergents used for cell lysis are: sodium dodecyl sulphate (SDS) and Triton X-100. Whereas the first one is a strong ionic detergent and tends to denature proteins from the cell, the second one is a mild non-ionic detergent that can be used e.g. to determine an activity of extracted proteins.

Results

In this study we describe a microfluidic system for a chemical cell lysis process. The microdevice was fabricated in poly(dimethylsiloxane) (PDMS) using photolithography and replica molding techniques [9,10]. A schematic view of the microdevice is presented in Fig. 2. The design of chemical cell lysis module is based on the *sheath flow* geometry [11]. This solution makes possible to obtain a maximum efficiency of a cell lysis process because each introduced to the microsystem cell is treated by the detergent from both sides.

There are three microchannels (each 120 μm wide and 50 μm deep), i.e. two side-focusing streams used for lysis buffer and the middle one for cell suspension. These three microchannels merge into a single channel (width of 300 μm and depth of 50 μm), in which the lysis process and the analytical enzymatic reaction may undergo simultaneously.

Investigations were performed using L929 fibroblasts. Their diameter of 12–13 μm limited the width of the middle (focused) stream with the cell suspension. The flow rates ratio of fluids introduced into the microdevice were investigated and optimized. Comsol Multiphisics 3.3 software was used for flow rates simulations of focusing process. Obtained results were verified by the measurements of fluorescence intensity profiles in streams' junction zone. The required middle stream's width of 15 μm was obtained by applying flow rates of the lysis buffer and cell suspension/substrate mixture in the ratio of 12:1, respectively. Almost 100% efficiency of cell lysis process in microsystem presented was confirmed by a viability test with propidium iodide. Nuclei of cells with disintegrated membranes are stained immediately, what it easy to observe under a microscope.

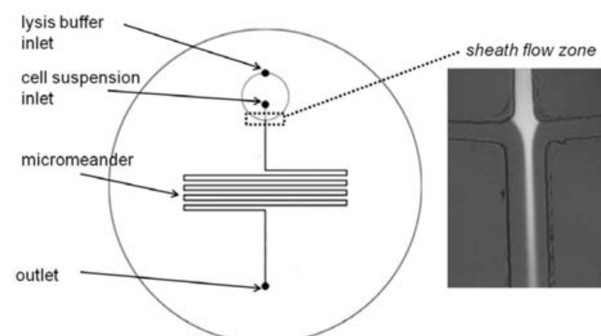

Fig. 2. A schematic view of the microdevice for a chemical cell lysis process.

Conclusions

The purpose of this work was to design and develop a microdevice for a chemical cell lysis process. The goal was achieved. Almost 100% of introduced to the microsystem cells were lysed. The main advantage of presented system and developed method is using non-denaturing cell lysis buffer, what enables to perform some investigations, i.e. enzyme assays just after cell lysis process. The effect of proteases released in a lysis process on determined enzymes was highly limited, what is difficult to achieve during macro-scale measurements.

Acknowledgments

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