Study of droplet formation regimes in a pressure control mode in microfluidic chip for screening cell libraries

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Abstract. To create a high throughput system for screening single cells in “water-in-oil” droplets, a key role is played by the method of controlling fluid flows in a microfluidic droplet generator. This is due to the fact that it is required to obtain monodisperse drops-microcapsules, place cells in them and be able to manipulate them. For these purposes, not only systems based on syringe pumps are suitable, but also pressure control units may be used. Unfortunately, pressure control instruments on the market for researchers are represented by only a few companies and are quite expensive. In this work, we develop a homemade microfluidic pressure controller based on SMC ITV 0010 pneumatic regulators and show its operability. Also, we investigated the conditions for reproducible generation of water-in-oil emulsions in a microfluidic flow focusing droplet generator by pressure driven fluid flows.

1. Introduction
In the middle of the 20th century, it was first proposed to study individual cells located in aqueous drops in oil [1]. However, only the development of droplet microfluidics allowed to achieve a stable and reproducible formation of monodisperse drops with a volume of 1-100 pl., suitable for quantitative research. Microfluidics enables to generate stable emulsions with controlled sizes and with high forming rate (> 1-10 kHz) to perform a large number of experiments. Also, it is possible to create conditions for the isolation of single cells in drops by generating emulsions of small volumes [2]. In contrast to the classical methods, this provides a huge potential for efficient cell screening [3]. Moreover, microdrops are in demand for applications on creation of biosensors [4], artificial cells [5], for investigating of protein crystallization [6] and manufacturing of bio-ink for 3D bioprinting [7]. Also, it is possible to immobilize living microorganisms in microdrops, such as fungus and worms for their detailed study [8,9].

To create a system for screening cells in a microfluidic chip, a key role is played by the method of controlling fluid flows to form droplets. This is due to the fact that it is required to obtain monodisperse drops-microcapsules, place cells in them and be able to manipulate them. As a simple solution and commercially available tools, syringe pumps are usually used to solve these aims. However, syringe pumps are typically operating open loop, specifying a particular rate at which the syringe is actuated, directly controlling the flow rate through the chip. While the mean flow rate for a period of time can be very accurate using syringe pumps, the transient flow through a system is pulsed, with the pressure fluctuating over time within a chip. Additionally, syringe pumps have long response times, which limit their use in microfluidic studies requiring dynamic flow profiles [10,11]. They are also incompatible with a valve-based closing system and closed microchannels. At the same
time, there are suitable alternative methods for controlling pressure of liquids, which have been little studied in application with systems for screening cells in microchips.

The aim of this work is to study the conditions for reproducible generation of water-in-oil emulsions in the microfluidic chip by the pressure control mode of fluid flows.

2. Methods

In experimental studies we have used microfluidic chips (figure 1) from polydimethylsiloxane (PDMS) and glass, manufactured by the "soft lithography" method [12]. The formation of the emulsion was carried out as follows: dispersed phase (aqueous medium) was supplied through the central channel, and continuous phase (mineral oil) - through two side channels, thereby compressing and focusing the flow of the dispersed phase. In specific hydrodynamic conditions due to instabilities, this leads to the formation of the emulsion [13]. By controlling the ratio of pressures of the dispersed and continuous phases, it is possible to obtain an emulsion with desired characteristics (for example, droplet diameter). To predict a coalescence of the emulsion, the presence of a surfactant in oil is necessary. Mineral oil (330779 light, Sigma-Aldrich) with a surfactant (Abil EM 180) was used as a continuous medium.

To understand the conditions of droplet formation, we chose a flow focusing droplet generator (figure 2) with asymmetric inputs. To manage fluid flows, a homemade pressure controller (figure 1b) based on SMC ITV00X0 series electro-pneumatic regulators with electromagnetic actuation was developed. The regulators differ in operating pressure range at the inlet and outlet. In this work, we used SMC ITV-0010, which works with a pressure range from 0.1 – 0.2 MPa at its inlet from an air compressor and adjust an output pressure from 0.001 to 0.1 MPa. Regulators have built-in pressure sensors with 6% accuracy and adjust output pressure depending on the voltage applied to the control pin. Therefore, to control the output pressure we used a microcontroller Arduino Mega 2560 with Adafruit MCP4725 digital-to-analog (DAC) converters. To communicate with the DACs, I2C protocol was used. To operate the unit from a computer, the software on Lab View was developed.

![Figure 1](image_url)

Figure 1. The scheme of the experimental installation for the formation of the monodisperse emulsion (a). Microfluidic pressure controller image (b). Sealed pneumatic interfaces image (c). Microchip image. To indicate microchannels the blue dye was used (d).
Since the formation of droplets is affected not only by the pressure of the flows, but also by the geometry of the channels, we used microfluidic chips with a microchannel height ~45 μm. The generator aperture \( a \) was 15 μm (figure 2b), the input channel width was 30 μm (near the place of drops breakaway). But we varied the output channel width: \( w = 60 \) μm or \( w = 200 \) μm. To test our technique for packaging single cells into drops, a model cell line of human chronic myeloid leukemia K562 was used.

### 3. Results and discussion

The homemade microfluidic controller allowed forming a macroemulsion with a droplet diameter from 10 μm to 110 μm. During experiments, compressed air with a desired pressure from the controller entered the containers (eppendorf 1.5 ml tubes) with dispersed and continuous phases through sealed pneumatic interfaces (figure 1c). Such interfaces were made by 3D printing using fused deposition modeling (FDM) technology. A PDMS gasket was used to seal fittings and capillary. Liquids from the pneumatic interfaces entered through the capillaries into the microfluidic chip. Then droplet generation was observed in the microchip by Leica DM 4000B LED microscope with a Pike F-100 B camera (Allied Vision Technologies).

To determine the optimal conditions for the droplet formation, the dependences of their diameter from pressure phases’ ratio were obtained (figure 3). During the study, more than 20 microchips were used. Stable droplet formation occurred under certain pressures in each chip.

![Flow focusing microfluidic droplet generator with asymmetric inlets: (a) single-row drops formation; (b) two-row formation; (c) Produced monodisperse macroemulsion.](image)

Controlled monodispersed generation (CV<9%) was observed at the water to oil pressure ratio range from 0.6 to 1.1. It was found that the droplet size of the emulsions lies in the range from 10 μm to 110 μm. The amount of the surfactant significantly affects the size of emulsions. If the surfactant concentration increase to 5% then the droplets diameter can rise. When the output channel’s width was \( w = 60 \) μm single row droplet generation was observed, although when the width was \( w = 200 \) μm the droplet formation occurred in several rows (figure 2a,b).

The experimental data of the dependence of the diameter of water emulsions in mineral oil on the ratio of water to oil pressures at the different output width showed that the diameter of the emulsions is independent on absolute water and oil pressures for \( w = 60 \) μm, but depends only on their ratio. In contrast to this, for \( w = 200 \) μm, the diameter also depends on absolute water and oil pressures.

Additionally we have tested our technique for packaging single myeloma cells K562 into the drops. The experiments have shown that the number of cells in the droplets is described by Poisson statistics with good accuracy.
4. Conclusions

To sum up, we have developed the pressure controller based on commercially available electro-pneumatic regulators and Arduino electronic boards and modules for the reproducible generation of water-in-oil emulsions with drop diameter in range from 10 – 110 µm in microfluidic flow focusing droplet generators with asymmetric inlets. Controlled generation was observed at the water/oil pressure ratio range from 0.6 to 1.1. The droplet diameter is highly dependent on the pressure ratio of the phases. Moreover, the droplet diameter for the narrow output channel (w = 60 µm) does not depend on the absolute values of the phases’ pressures, in contrast to the wide outlet (w = 200 µm). This can be caused not only by the microchannel width but also by asymmetric inputs. Thus, the geometry of the channels can greatly affect the pressure ranges of possible stable generation. In addition, the ability to encapsulate K562 cells in droplets was tested and Poisson statistics of cell distribution was confirmed.

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