Formation and manipulation of polyacrylamide spheroids doped with magnetic nanoparticles in microfluidic chip

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Abstract. Nowadays isolation and sorting of biological objects plays an important role in life science and medicine. Magnetic sorting has big opportunities because of its advantages such as sufficient selectivity and bioinerticity. In this work we studied formation and handling of polyacrylamide microparticles doped with magnetic nanoparticles using droplet microfluidics. Using a flow focusing droplet generator magnetic nanoparticles were encapsulated to polyacrylamide droplets which were polymerized inside the device. It was shown that these magnetic droplets could be efficiently sorted by magnetic field in a microfluidic device.

1. Introduction

Nowadays precise sorting became one of the significant objectives in biology and medicine. It is essential for separation, isolation and analysis of defined types of biological objects such as cells, vesicles, exosomes, DNA molecules, peptides and others with defined properties (productivity, size, specificity, etc.). Different sorting techniques can be divided into three trends: optical (imaging techniques and optical-detected signals like FACS [1]), field-based (magnetophoresis [2], electro- or dielectrophoresis [3]) and mechanical (hydrodynamic sorting or deterministic lateral displacement method [4]). Utilization of magnetic sorting has several advantages: it is biologically non-invasive and doesn’t cause harmful effects on biological objects.

Microfluidics is ideally suited for handling biological objects and new approaches have become increasingly accessible for researchers and clinicians. Magnetophoretic sorter can be implemented in microfluidic devices, which leads to reducing its size, cost and complexity. Due to small sizes of the device ordinary permanent neodymium magnets can be used to create strong magnetic fields for efficient separation.

Biological objects usually have weak magnetic properties, magnetic particles can be used to enhance magnetic sorting’s efficiency. Especially, superparamagnetic nanoparticles are highly suitable for such application, because of there one-domain structure and zero coercivity, leading to lack of residual magnetization and absence of motion without any external initiation [5]. For molecular magnetic sorting these nanoparticles can be conjugated with antibodies or oligonucleotides, to which target molecules will be attached [6]. For cell sorting, magnetic particles can be affixed on the cell’s membrane with a targeting ligand (extrinsic labeling), or they can get through a membrane and accumulates inside a cell (internalization via cell encapsulation) [7].

Due to small size and potential toxicity and instability of superparamagnetic nanoparticles in biological environment the common way is to encapsulate them into polymeric micro or nanoparticles. The surface of such containers can be labeled with target molecules for isolation and sorting of
exosomes, proteins or DNA molecules, which can be attached the container’s modified surface and then removed from the sample.

Recently droplet microfluidics was introduced as a high throughput technique to produce monodisperse polymeric and hydrogel particles with defined properties [8]. In this work, we studied encapsulation of magnetic nanoparticles into polyacrylamide droplets and investigated their suitability for magnetic sorting in a microfluidic device.

2. Materials and methods.

For generation of polyacrylamide droplets microfluidic chip with a flow-focusing droplet generator and twisted outlet channel (Figure 1a) was used. Its operating principle is based on the fact that the continuous phase flowing from two side channels meets the dispersed phase at the channel’s intersection, where the dispersed phase get squeezed by the continuous phase and breaks up into droplets (Figure 1b). Droplet size can be precisely controlled with changing the flow rates of the liquids. For magnetic separation another microfluidic device with long strait channel and two outlets was used.

Microfluidic devices were fabricated using standard soft lithography process from PDMS Sylgard 184 (Dow Corning) [9]. The droplet formation region represents the intersection of two channels for continuous phase and one channel for dispersed phase, separated from the outlet channel by 15 μm aperture. Width of the outlet channel near the aperture is 60 μm. Depth of all inlet channels, droplet formation region and the outlet channel near the aperture is 40 μm. The twisted channel was manufactured wider and deeper (120 μm x 120 μm) than generator’s channel to prevent sticking of the polymerized particles.

Figure 1. a) General view of a microfluidic flow focusing droplet generator with twisted outlet channel; b) Generation of polyacrylamide droplets in a flow-focusing droplet generator

Mineral light oil (cat. N. 330779, Sigma Aldrich) with 4% w/w ABIL EM 180 surfactant (Evonik Industries) and catalyst for polymerization – 1.5% w/v TEMED (Sigma Aldrich) were used as a continuous phase. The mix of 30% acrylamide solution (Bio-Rad), initiator for polymerization - 10% water solution of ammonium persulfate (BioRad) and 0.1% of ferrofluid (magnetite nanoparticles, 400 mg/ml aqueous solution) was used as a dispersed phase. The size distribution of magnetic iron oxide nanoparticles was measured by dynamic light scattering method using Zetasizer Nano ZS (Malvern Instruments, UK).

After the generation, droplets were re-injected to the second microfluidic device with long strait channel for magnetic sorting. To cause lateral displacement of droplets, permanent rectangular NdFeB magnet (N38 alloy) was used.

For introducing continuous and dispersed phases into the microfluidic chip the microfluidic pressure controller based on ITV001 electro-pneumatic regulators (SMC, Japan) was made. The chip was observed using optical microscope Leica DM4000 B LED (Leica Microsystems). Droplets generation and motion were recorded using a camera Pike F100B (Allied Vision Technologies).

3. Results

In our case, the magnetic force acts on every droplet [10]:

\[ F = \frac{x_e \times x_e}{\mu_0} V(B \nabla) \]
where \( \chi_p \) - magnetic susceptibility of droplet with magnetic nanoparticles, \( \chi_e \) - magnetic susceptibility of environment, \( V \) - volume of the droplet (m³), \( \mu_0 \) is the magnetic constant (H m⁻¹), \( B \) - magnetic flux density (T). Also, the Stokes drag acts in the opposite direction to magnetic force:

\[
F_d = 6\pi \eta r v,
\]

where \( \eta \) is the viscosity of oil (kg·m⁻¹·s⁻¹), \( r \) - the radius of the droplet (m), \( v \) - droplet velocity in displacement direction (m·s⁻¹). Both of this forces tends to lateral displacement:

\[
L \sim \frac{(\chi_p - \chi_e) V^2 \mu_0 B^2}{6 \pi \eta r \mu_0}.
\]

Based on these formulas, droplet velocity and time, that droplet with magnetic particles requires for relocation from one sidewall of the channel to another under the influence of magnetic force, the length of a sorter’s magnetic force zone was set to 4 mm: dimensions of magnet satisfied this parameter.

Firstly, the size distribution of magnetic nanoparticles was obtained by using dynamic light scattering method in diluted ferrofluid. Consequently, by 14 measurements the size of nanoparticles was defined in range of 35 -145 nm (Figure 2a). Then, nanoparticles were packed into droplets. Due to microfluidic way of formation, size distribution of droplets is narrow. Because of stochastic loading of droplets with magnetic nanoparticles, the probability of packing one determined amount of nanoparticles can be described with Poisson statistics, where the distribution parameter

\[
\lambda = \frac{\text{quantity of nanoparticles}}{\text{quantity of droplets}} \sim 5 \cdot 10^3
\]

is very high, so it can be approximated by Gauss distribution with \( \sigma \approx \sqrt{\lambda} \).

Utilization of above-mentioned topology of flow-focusing droplet generator allows to produce droplets with diameters from 15 μm to 80 μm in dripping mode inside the generator channel and up to 100 μm in jetting mode, when stream of dispersed phase reaches wide outlet channel. In this work, droplets with 40-50 μm diameter were used (Figure 2b), which were generated in dripping mode. Acrylamide polymerization was initiated by TEMED diffusion from continues phase inside droplets after their formation. Twisted outlet channel of droplet generator increases the time that droplets stay in the chip, so acrylamide solution turns into gel inside it and on the way out the suspension of solid beads was collected.

The next step is to introduce magnetic gel beads into the second chip, which has a straight separation channel and two outlet channels with different hydrodynamic resistance (~1:2.5). So without any external forces, beads with magnetic particles will flow into a channel with less resistance. Droplet flow was focused to the upper wall of the chip near the entrance to the separation channel (Figure 3a) and guided to the most probable outlet (Figure 3b). In our case, velocity of droplet motion along the channel is about 200 μm/s
After that, the permanent magnet 30x5x5 mm with magnetization, perpendicular to channel’s axis was used to organize a lateral displacement of droplets. As a result, droplets were guided by magnetic force into less probable channel with higher hydrodynamic resistance (Figure 3c) with speed about of 25 μm/s.

**Figure 3.** a) Focusing of droplet flow; b) Droplets motion without magnet; c) Droplet motion with magnet, scale bars are equal to 100 μm.

4. Conclusions
The suspension of magnetic-modified polyacrylamide beads with narrow size distribution was produced in flow-focusing microfluidic device with on-chip polymerization. The possibility of using permanent magnet to manipulate this beads was shown, what presents the suitability of polyacrylamide beads for magnetic on-chip sorting.

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