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Microfluidic preconcentrator and microfluidic chip for bacterial cells detection

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Flow cytometry is a very popular clinical diagnostic method for a fast analysis of different kinds of cells/microparticles. In classic cytometers fluorescence, labeled cells are hydrodynamically focused in flow stream to order them and direct individually to a detection area with an optical detector. After laser excitation, the fluorescence emission light is directed into a light sensitive window of an optical detector (photomultiplier). Output signals of the detector may be counted in the digital or analog form (integration). In the experimental system, the photomultiplier was used as the integrator, so its output voltage was proportional to the number of fluorescence labeled cells. To miniaturize the flow cytometer, own technology for fabrication of microfluidic structure as a pre-concentrator with utilization of SU-8 masters was used.

Keywords: micro flow cytometer, poly(dimethylsiloxane) – PDMS, optical detection.

1. Introduction

In classical flow cytometers, the analytical process starts with the introduction of a sample, *i.e.*, cells or microparticles suspension, into the fluidic system consisting of concentric tubes, where the inner tube is shorter than the external one. The sample enters through the inner tube while the sheath fluid through the external one. Thanks to proper ratio between flow rates in both tubes, the sample is hydrodynamically focused and the cells/microparticles move in an ordered line, to the detection area. There, fluorescent dyes labeling cells or microparticles are excited by laser light, and next emitted light is detected by a detector, *e.g.*, photomultiplier (Fig. 1). The intensity of light emitted by fluorochrome is proportional to its concentration. This reflects the properties or the number of analyzed cells (or other particles) [1, 2].

In cytometry, the most often used light sources are lasers of the following wavelengths: 458, 488 or 514 nm and power in a range 5–75 mW. A very important

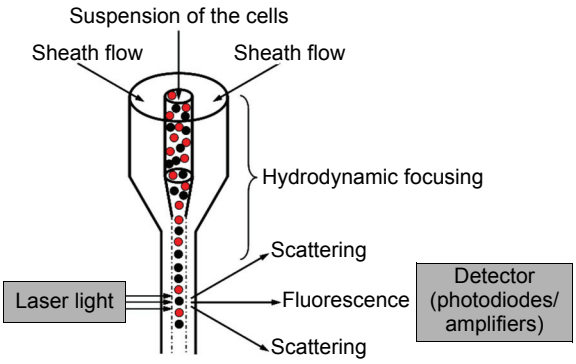

Fig. 1. The principle of operation of flow cytometry.

problem is a selection of the suitable fluorochrome or fluorochrome-labeled antibody. The Table shows fluorochromes commonly used in the flow cytometry.

Recently, many research groups work on miniaturization of flow cytometers [3–7]. A new kind of this device will become portable, cheaper and of low power. Moreover, it would be possible to design a modular system, in particular combine several microfluidic devices for multitask applications [8]. In order to construct this kind of chips, a poly(dimethylsiloxane) – PDMS – is commonly used. This elastomeric substance has many advantages. It is biocompatible, inexpensive as well as transparent in a wide range of light wavelength and applicable for patterning with dimensional accuracy of about 0.1 μm. The PDMS (Sylgard 184, Dow Corning) is a composition of two liquid ingredients: monomer with vinyl terminated siloxane chains and curing agent–siloxane chains with silica hydrogens (Fig. 2).

T a b l e. Fluorochromes commonly used in flow cytometry.

Fluorochrome	Excitation wavelength [nm]	Emission wavelength [nm]
Propidium iodide (PI)	488	617
Texas-red (PE)	488	615
Fluorescein isocyanate (FITC)	488	518
Pacific Blue®	405	455

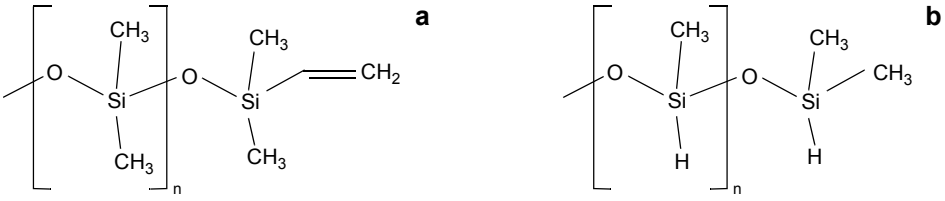

Fig. 2. Structural formulas of PDMS monomer (**a**) and its curing agent (**b**).

2. Fabrication of PDMS microfluidic systems

Technological processing of microfluidic PDMS structures consists of several steps. The first one is formation of templates for a microfluidic structure.

The SU-8 templates for molding were fabricated from epoxy-type negative photoresist SU-8 (MicroChem) (Fig. 3) on silicon substrates. Silicon 4" wafers were cleaned in piranha solution (H_2SO_4, H_2O_2) and thermally oxidized ($xSiO_2$ = = 500–1200 Å), to improve adhesion of the photoresist to the substrate. The first thin 15–20 μm polymeric SU-8/25 layer was spin-coated on a silicon wafer using Convac spin-coater 500(5 s)/2600–2620(30 s), planarized, soft-backed, exposed to UV light (365 nm, 200 mJ/cm^2) and post-exposure backed (PEB). Next, constructive layer was fabricated from SU-8/50, to obtain 70 μm thick structures. The photoresist was spin--coated with the following sequence of velocities 500(10 s)/1500(30 s), planarized, soft-backed, exposed to UV light (365 nm, 350 mJ/cm^2) and post-exposure backed.

As it was mentioned, the SU-8 layer is exposed to UV light through the patterned photomask. SU-8 is cross-linked in the areas exposed to the UV light only. After this stage, non-cross-linked photoresist is removed with a special SU-8 developer (MicroChem). In effect, the master silicon wafers with negative pattern of the micro-fluidic structures are obtained (Fig. 4) [9].

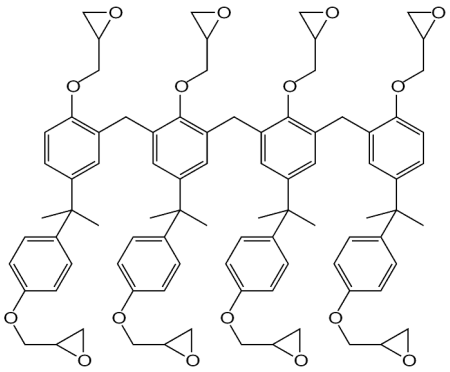

Fig. 3. Structural formula of the SU-8 resin.

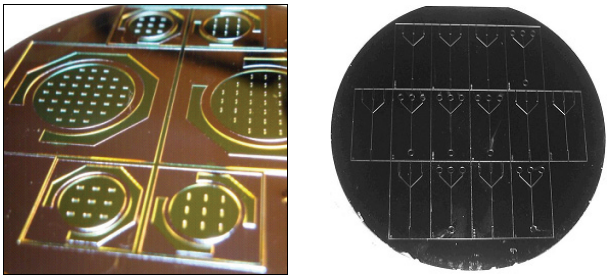

Fig. 4. Masters of a pre-concentrator and microfluidic chip, with negative patterns of microchannels made in UV-curable epoxy resin SU-8.

The next stage consists in PDMS structure formation. Firstly, PDMS pre-polymer was prepared by mixing PDMS monomer and curing agent in 10:1 weight ratio. Next, the pre-polymer was degassed, and then molding process using the SU-8/silicon wafer master with negative pattern of the microchannels (Fig. 4) is performed.

The pre-polymer is cross-linked at 70 °C for 2 hours. Mechanism of this radical reaction depends on formation of ethylene bridges between the monomer and chains of the curing agent (Fig. 5).

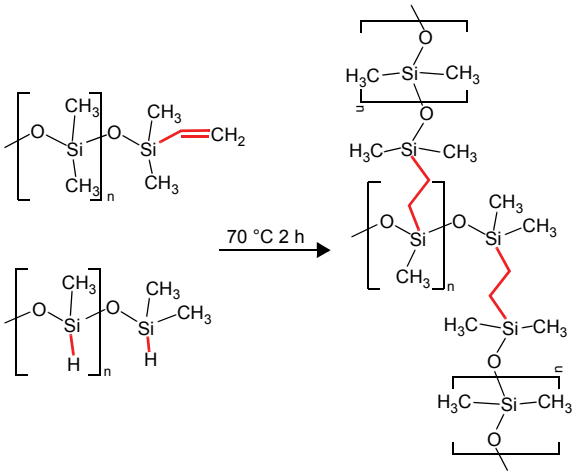

Fig. 5. Crosslinking reaction between PDMS monomer and curing agent.

After separation of a cross-linked PDMS plate from the master, PDMS planar (non-patterned) and patterned plates were activated with oxygen plasma. The last step consists in connecting the PDMS plates and heating at 80 °C for 2 hours [10–13].

3. Pre-concentrator and microfluidic chip

As described above, miniaturized cytometers can be combined with other microfluidic components. Our analytical system is two-part, namely a pre-concentrator and chip for bacterial detection.

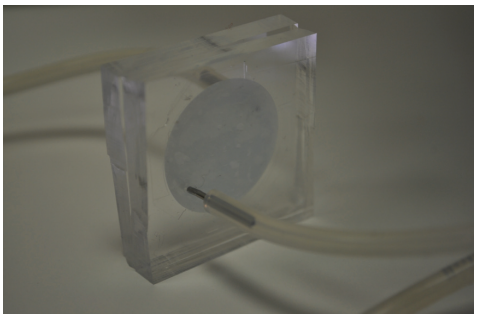

Fig. 6. PDMS pre-concentrator with polycarbonate filtration membrane and microfluidic connectors.

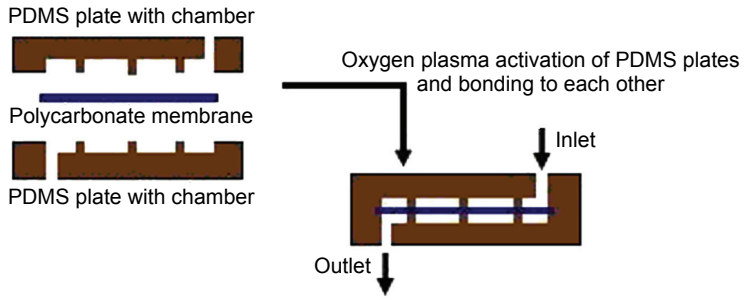

Fig. 7. Method of fixing of polycarbonate membrane inside the pre-concentrator.

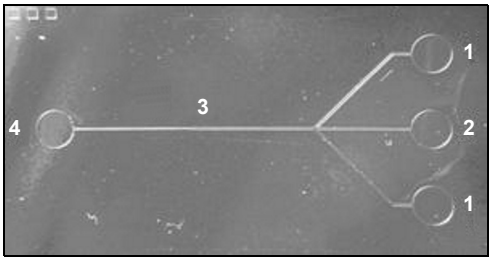

Fig. 8. PDMS microfluidic chip.

The pre-concentrator consists of two PDMS plates with chambers and a polycarbonate membrane filter (0.22 μm porous or other) placed between them (Fig. 6).

In Figure 7 a fixing method of the polycarbonate membrane inside the pre-concentrator is shown.

The pre-concentrator has a very important function, namely filtering impurities from the target cells/microparticles. This operation allows to increase purity of the sample, decrease interferences and collect analyzed material in a small-volume sample (pre-concentration).

After filtration in the pre-concentrator, microparticles to be analyzed are washed from the surface of the membrane to the tank and labeled by the fluorochrome. Then, the prepared analytical material is directed to the inlet 2 of the microfluidic chip (Fig. 8). Simultaneously, the sheath fluid is introduced to the inlets 1. The focused sample stream is analyzed in the detection area 3.

4. Experimental results

At first, filtration properties of the pre-concentrator were tested. For this purpose, water suspension of the calcium carbonate was used and absorbance of the suspension was measured before and after its travel through the pre-concentrator. Results of this experiment are presented in Fig. 9. As can be seen, the absorbance after filtration is much lower than before this process. This proofs very good filtration properties of the pre-concentrator and no-leakage of the sample around the membrane.

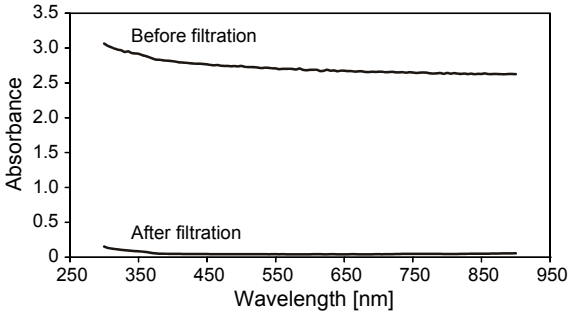

Fig. 9. Absorbance of water suspension of calcium carbonate before and after filtration in the pre-concentrator.

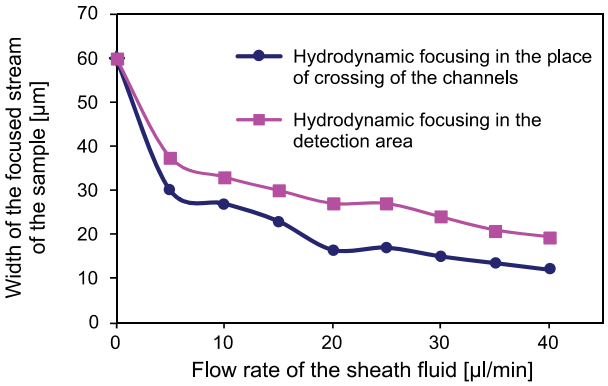

Fig. 10. Relationship between flow rate of sheath fluid and width of the central fluid stream after hydrodynamic focusing; flow rate of the central fluid – 10 µl/min.

We also investigated focusing of the central fluid in the microchannel of a microfluidic chip. Figure 10 displays relationships between the flow rate of sheath fluid and the width of the sample (central fluid) stream after hydrodynamic focusing in two cross-sections of the microchannel (crossing of the channels and in the detection area), when the central fluid flow is 10 µl/min.

5. Conclusions

Presented technology of fabrication of PDMS microfluidic structures was developed. The obtained pre-concentrator has a good filterability and a hermetic seal. Moreover, in the developed microfluidic chip (as it was expected), width of the focused stream depends on sheath fluid flow rate and it decreases with the increase in the flow rate of the sheath fluid.

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