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Elastomer planar device for nucleic acids extraction

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Abstract. In this paper a device that provides the nucleic acids extraction and loading into a microfluidic chip for subsequent analysis by amplification methods was performed. The device is made by soft lithography from Silastic T4. The mechanical properties of the material (Young's modulus) and the contact angle after Ar plasma treatment were studied. The strength of the connection of the device elements was evaluated. The device usability was checked by nucleic acids extraction from *E.coli* samples and using model solutions of the GAPDH gene cDNA.

1. Introduction

Sample preparation of genetic material from environmental samples remains one of the most difficult and time-consuming stages for sample analysis by amplification methods. The standard protocol for the nucleic acids extraction includes such steps as sample purification from impurities, lysis, extraction and concentration of the target product. An important task in instrumentation engineering is to automate and combine all stages of sample preparation and analysis in one device [1, 2], that can reduce the impact of the human agency on the obtained results, increase the reliability of the analysis and reduce its time.

In this work a device for the nucleic acids extraction and loading into a microfluidic chip for subsequent analysis by amplification methods (qPCR, isothermal) was performed. The device is a planar structure with a system of sealed serpentine channels (for sample lysis, nucleic acids extraction and concentration) and ports for connection to external microcells (containers) and a microfluidic device (MFD). The fluid motion in the channels is controlled using an external pump and valves. Nucleic acids are extracted from the lysate using magnetic beads. The device allows you to work with large volumes of the initial sample because the sample and reagents are loaded from external containers.

One of the most widespread and well-studied materials in microfluidics is polydimethylsiloxane Sylgard-184 (Dow Corning). However, its downsides are porosity and low resistance to alcohols and chlorine-containing compounds. So the device components are obtained by "soft" lithography from Silastic T4 elastomer (Dow Corning). Since the material has never used for creating microfluidic devices but in the same time does not inhibit PCR [3] and is less porous than Sylgard-184, we studied the possibility of using it for prototyping of microdevices.

2. Materials and methods

The mechanical properties of the Silastic T4 elastomer (Young's modulus) were studied by the method described in [4]. Since the elastomer has a high viscosity that can affect the accuracy of obtaining



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replicas, the reduction of the viscosity by adding a diluent was studied. The elastic modulus was also measured.

To select a mode that provides stability of the material wettability properties after argon plasma treatment, the material was measured using the sessile drop technique. Measurements were performed after argon plasma treatment, first every hour (7 hours), then once a day. Three measurements were performed on each sample.

To evaluate the strength of the structural elements connection after plasma treatment round chambers with a diameter of 5 mm were made. Such device, after a tight connection, was placed in a rigid mandrel consisting of two plates connected by screws. On one side there was a fitting for supplying air under pressure in the center of the chamber, and on the other there was a slot with a 12 mm diameter for not preventing the connection from breaking.

The nucleic acids extraction device was made of Silastic T4 elastomer (Dow Corning) using soft lithography technique. Replicas were obtained using a master mold made of aluminum alloy D16 by milling. A plate made of the same material was used as a protective one. Sealed connection of the replica and the protective plate was provided after their plasma treatment in argon for 1 minute (Zepto, Diener electronic, Germany). The device configuration was designed to use with a set of reagents "M-Sorb-OOM" (Syntol, Russia) for the DNA and RNA extraction from clinical samples and environmental objects according to the manufacturer's methodology. Fluid motion was controlled using an external BT100-2J peristaltic pump (LongerPump, China) and valves. Heating for lysis, drying of magnetic beads with a sample and elution were carried out by a heating device based on a planar heating element EO-20 "Developer's kit" (Nicole, Russia).

The study of nucleic acids extraction from a biological sample in a microfluidic device was carried out using model objects: *E. coli*, cDNA fragment of the GAPDH gene (226 b.p.). Analysis of samples after extraction was performed by qPCR at ANC-48 (IAI RAS). For experiments the reaction mixture M-428 "PCR-Mix" (Syntol, Russia), specific primers with probes for *E. coli* (Syntol, Russia) and the GAPDH housekeeping genes (DNA Synthesis, Russia) were used.

3. Experiment

3.1. Investigation of Silastic T4 properties

Plasma treatment was used for device sealing so a study of its effect on the wettability of the surface by sessile drop technique was carried out. After treatment some samples were left at room temperature, some were placed in a thermostat at 60°C, and some at 80°C. For the Silastic T4 initial material, the contact angle was 103±2 degrees. After argon plasma treatment the angle value became less than 10 degrees. For samples left at room temperature the hydrophobic properties of the surface were restored in 3 days after treatment. Temperature exposure can speed up the restoration of surface properties. Aging samples at 60°C for 7 hours led to a contact angle of 81.6±2.1°. Meanwhile, after keeping at room temperature for 2 days the angle reaches the initial value of 103 degrees. The temperature of 80°C can restore the properties of the surface in 7 hours.

Measurements of the Silastic T4 elastomer Young's modulus depending on the ratio of components and the concentration of the diluent are shown in Table 1. Increasing the curing temperature can faster the process of obtaining element from 24h to 60 min, almost without effect on the modulus of elasticity. However, doubling the amount of curing agent leads to an increase of the Young's modulus by 0.1 MPa. Adding a diluent simplifies the degassing process, but reduces the rigidity of the elements.

The diluent addition also reduces the connection strength of manufactured elements with each other after argon plasma treatment. The chambers from the initial material withstand a pressure of 350 MPa (each second breaks at 370 kPa), but adding 5% of the diluent leads to the break of half of the chambers at 200 kPa, 10% of the solvent – they withstand 100 kPa, they all break at 200 kPa.

Table 1. Dependence of Young's modulus on the ratio of Silastic T4 components

Composition		Curing temperature, °C	Young's Modulus, MPa
Base : Curing agent	Diluent		
10:1	-	24	1,90±0,04
10:1	-	40	1,91±0,03
10:2	-	40	2,01±0,09
10:1	5	40	1,79±0,09
10:1	10	40	1,55±0,13

3.2. Nucleic acids extraction

The scheme and image of the nucleic acid extraction device are shown in Figure 1. Automatic extraction was performed as follow: 100 µl of samples mixed with a lysing solution and a lysing component were inserted through the sample input port into the serpentine channel for lysis and kept there for 15 minutes at a temperature of 65°C. Then through the extraction port the magnetic beads diluted by distilled water were pumped in the serpentine extraction channel and were kept there by a magnet. Water was removed from the device's channel through the pump port. Further the sorbing solution was loaded into the inlet of the extraction port. A vacuum was created at the pump connection port so a lysate mixing with the sorbing solution came to the magnetic beads. For better mixing the liquids were pumped through the extraction channel several times in reverse mode. The lysate passed through the magnetic beads three times to provide good nucleic acids sorption. Depended on the direction of the liquid flow the magnet position was changed to provide better washing of magnetic beads with nucleic acids by flow. After sorption the reagents remains were removed from the device channels through a pump port. Thereafter the magnetic beads with sorbed cDNA were washed three times in the same way. Despite the complex configuration of the microfluidic device prevents effective drying of beads, the air were pumped through the channel at the maximum pump speed for 15 minutes at a temperature of 65°C for better drying. After that 200 ml of the eluting solution was loaded through the extraction port into the channel with magnetic beads and the eluting solution was pumped several times through the magnetic beads in both directions at a temperature of 65°C. At the last stage the solution with the extracted nucleic acids was loaded through the extraction port to a microfluidic tube or microfluidic chip and then was analyzed by qPCR on the ANK-48 device. The device can be used with large volumes of sample and lysing reagents, but the research was carried out for 100 µl of sample. The eluting solution volume was selected 200 µl due to the large cross-section of the channels.

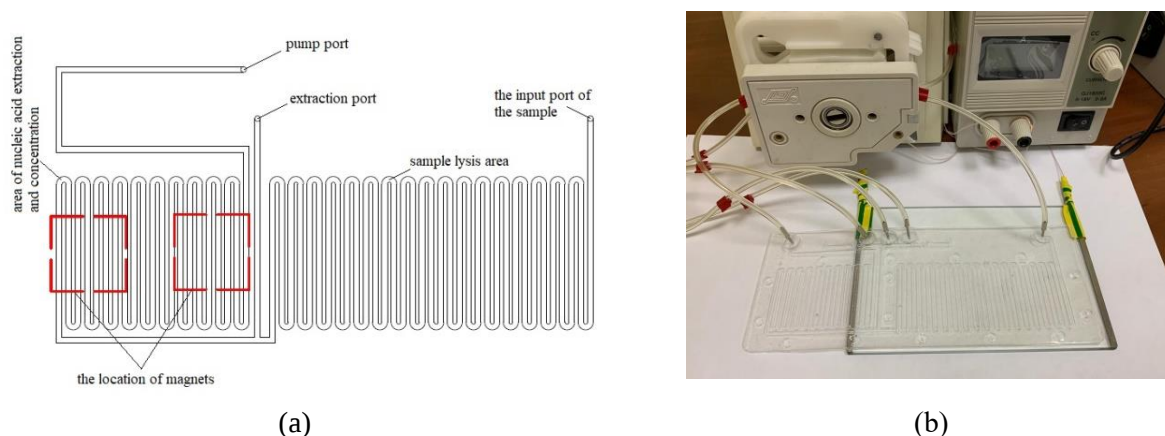


Figure 1. Sheme (a) and image (b) of the microfluidic planar device

Manual extraction was performed according to the instructions of the set "M-Sorb-OOM".

The values of qPCR threshold cycles of DNA extraction from *E. coli* are shown in Table 2. The difference in the threshold cycles for the microfluidic device (with a dilution of 2 times by the method) and in test tubes (without dilution) was 1,2 cycles. To increase the efficiency of nucleic acid extraction in a microfluidic device, it is necessary to optimize the method and modes of extraction.

Table 2. Threshold cycles after *E. coli* cell DNA lysis and extraction in a test tube and microfluidic device

№	Description	Dilution	Threshold cycle
1	manual extraction in a test tube	without dilution	23,4
2	extraction in the device	2 times due to the volume of the eluting solution	24,6
3	extraction in the device	4 times	26,4
4	extraction in the device	10 times	27,6

To evaluate the nucleic acid extraction efficiency in a microfluidic device, experiments were carried out with model solutions of the GAPDH cDNA gene of known concentrations.

Table 3 and Figure 2 show the results of qPCR of the initial sample with concentrations of 10^2 , 10^3 , 10^4 and 10^5 copies/ μ l and also samples after manual extraction in a test tube and after extraction in a microfluidic device.

Table 3. Values of threshold cycles after manual extraction in a test tube and extraction in a microfluidic device of the GAPDH cDNA gene

N п/п	Initial concentration (copy/ μ l; cycle)	Manual extraction simulation, cycle	Device extraction simulation, cycle
1	10^5 ; 23,7	29,9	33,4
2	10^5 ; 23,9	24,1	26,1
3	10^4 ; 27,5	27,3	$29,05 \pm 0,07$
4	10^3 ; 30,8	30,8	$32,90 \pm 0,28$
5	10^2 ; 34,6	33,1	33,4

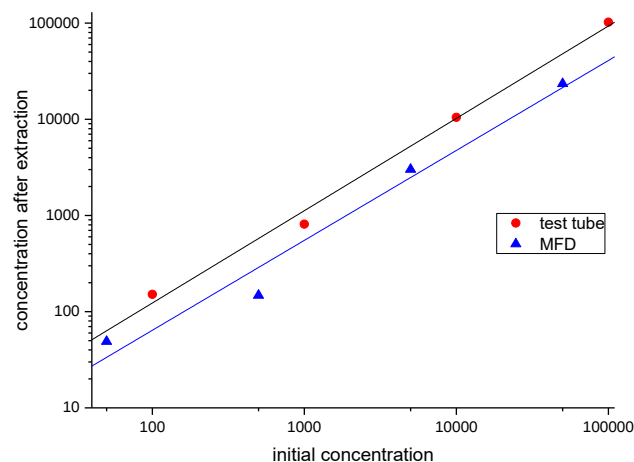


Figure 2. Experimental results of qPCR: the ratio of DNA concentrations in the initial solution and after extraction in a test tube (test tube) and in a microfluidic device (MFD) in logarithmic coordinates

In this series of experiments on a microfluidic device, lysis simulation was performed by mixing the lysing solution, the lysing component and 100 μl of the sample and mixing this solution in a serpentine channel for lysis at a flow rate of $\sim 6 \mu\text{l/s}$ and a temperature of 65°C . Further, the solution was mixed in the chamber with the precipitating one and pumped in both directions along the extraction channel through magnetic beads 5 times at a flow rate of $\sim 28 \mu\text{l/s}$. Similarly, three washings were performed. The elution was conducted at the same speed for 10 minutes according to the set instructions. On the extraction stage in a microfluidic device the sample was diluted twice during elution. Individual reagents were replaced with new ones during the repeat of extraction in a microfluidic device in the experiment with a concentration $10^3 \text{ copy}/\mu\text{l}$.

The obtained results can be approximated by linear dependencies:

$$C_{\text{tube}} = (0,96 \pm 0,05) \cdot C + (0,17 \pm 0,20);$$

$$C_{\text{MFD}} = (0,94 \pm 0,11) \cdot C - (0,06 \pm 0,37),$$

where C is the concentration of nucleic acids in the initial solution, C_{tube} is the concentration of nucleic acids after the simulation stage of "manual" extraction according to the standard instructions, C_{MFD} is the concentration of nucleic acids after the simulation stage of extraction in a microfluidic device.

Experimental data shows that the difference in the threshold cycle for the initial sample and after manual extraction is small (1.5 ± 0.8) and almost is non for the concentration of the initial sample of 10^3 and $10^2 \text{ copy}/\mu\text{l}$. The slope of linear approximations is close to 45° , but for the case of extraction in the device, the dependence is lower, which is probably due to the loss of sample on the working surface (in the channels) of the device. However, this hypothesis needs to be verified.

After nucleic acid extraction the channels of microfluidic devices first were washed with chlorine-containing solutions at a flow rate of $\sim 23 \mu\text{l/s}$ for 5 minutes and then 20 minutes with distilled water and dried at a temperature of 80°C . Then 200 μl of the eluting solution was cyclically pumped at the same flow rate through the sample separation channel for 10 minutes. Then the solution was monitored by qPCR. The value of the qPCR threshold cycle of this sample was 35,4, which reflecting the sorption of nucleic acids in the device channels.

4. Results

Restoration of the wettability properties of the Silastic T4 surface after argon plasma treatment was investigated. The contact angle of the initial material was 103 ± 2 degrees, but after processing - less than 10 degrees. Restoration of the material surface properties at room temperature occurs in 3 days, at a temperature of 80°C - in 7 hours.

Reducing the Silastic T4 viscosity can be achieved by adding a diluent. Adding of 5% of the diluent leads to a decrease in the Young's modulus from $(1,91 \pm 0,03)$ by 0.1 MPa, and 10% of the diluent - by 0.4 MPa. That, in turn, leads to a decrease in the parts joint strength after argon plasma treatment. So the chambers made from the initial material can withstand a pressure of 350 MPa, but after adding of 10% of the diluent 100 kPa.

A microfluidic device prototype for cell lysis and nucleic acids extraction on magnetic beads was developed and tested. Lysis of *E. coli* cells with subsequent nucleic acids extraction was shown. The difference between the extraction results obtained on the microfluidic device in automatic mode (with dilution by the method) and in "manual mode" test tubes (without dilution) and registered by qPCR was 1,2 cycles.

The efficiency of nucleic acid extraction in a microfluidic device was evaluated using model solutions of GAPDH cDNA gene of known concentrations of 10^2 , 10^3 , 10^4 and 10^5 copies/ μ l. The difference in the threshold cycle for the initial sample and after manual extraction is (1.5 ± 0.8) almost is non for the initial sample concentration of 10^3 and 10^2 copies/ μ l. The slope of linear approximations is close to 45° , but for the case of device extraction, the dependence is lower, which is probably due to the loss of sample on the working surface (in the channels) of the device.

The value of the qPCR threshold cycle for the eluting solution was 35.4 after washing the device channels that indicates the sorption of nucleic acids in the channels. It was shown that the developed device can withstand disinfection with chlorine-containing solutions.

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