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A microfluidic micromixer fabricated using polydimethylsiloxane-based platform for biomedical applications

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Abstract: Personalised dosing microfluidic devices have great potential in transforming current biomedical treatment into more efficient and patient-tailored using lab-on-chip designs. One of the current challenges in manufacturing microfluidic devices is designing suitable mixers, at the microscale level, with intricate geometrical dimensions. The study aimed at designing micromixers using polydimethylsiloxane-based platform and investigated their performance and potential applications in biomedical devices. New microchip-like structure was fabricated and consisted of two inlets and one outlet. A mould was fabricated based on polydimethylsiloxane platform and the new design was examined in terms of mixing patterns. The flow-mixing process was tested for efficiency and robustness. The novel design showed consistent intricate dimensions suggesting fabrication method was robust and precise. The mixing ability of the micromixers showed semi-circular flow with efficient mixing at low liquids pressure (< 50 mbar) suggesting ability to mix fluids with various viscosities. Accordingly, the newly designed micromixers using polydimethylsiloxane-based platform with two inlets and one outlet have promise in biomedical fluid-mixing applications.

Keywords: Micromixer, PDMS; Mixing efficiency

Mikrofluidični mikro mešalnik z uporabo platforme na osnovi polidimetilsiloksana za biomedicinske aplikacije

Izvleček: Mikrofluidične naprave s poosebljenim doziranjem imajo velik potencial pri spremembi trenutnega biomedicinskega zdravljenja v bolj učinkovito zdravljenje s pacientu prilagojenim dizajnom lab-on-chip naprav. Trenutni izziv je načrtovanje ustreznih mešalnikov na mikro nivoju. Članek predstavlja načrtovanje mikro mešalnikov z uporabo platforme na osnovi polidimetilsiloksana in njihovo integracijo v biomedicinske naprave. Nova mikročip struktura ima dva vhoda in en izhod. Kalup je izdelan na osnovi polidimetilsiloksana. Nov dizajn je bil opazovan s strain različnih mešalnih vzorcev. Proces pretočnega mešanja je bil preizkušen na osnovi učinkovitosti in robustnosti. Mešalna sposobnost kaže semi-cirkularni pretok z učinkovitim mešanjem pri nizkih tlakih (< 50 mbar) in različnih viskoznostih tekočin.

Ključne besede: mikro mešalnik, PDMS; izkoristek mešanja

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1 Introduction

The proper mixing of reagents is an important process in biochemical experiments, which aims to incorporate various fluids as a way to detect concentrations of biological molecules. Microfluidic devices have attracted a huge attention due to a wide range of potential applications such as in diagnostics and pharmaceutical and biological applications [1-3]. The purpose of microfluidic mixing is to obtain a complete and fast mixing of numerous samples in microscale devices, by employing the diffusion effect at low Reynolds number in microfluidic systems [4]. Microfluidic technologies can be used to produce micromixers in order to make the whole process more efficient and reliable, minimizing cross-contamination and reducing the time and labour costs. Mixing in microchannels is a principle applied in many modern biomedical devices. Active and passive mixing approaches can be found in microfluidic chips [5]. Active micromixers require external energy sources (electro-kinetic [6], [7], ultrasonic [8], electrostatic or magnetic fields [9, 10] to generate perturbations in fluid flow. These devices provide a very good mixing characteristics and flow control. However, they are expensive for fabrication and cannot be easily integrated into complete microfluidic systems. Passive micromixers depend on diffusion process [11]. A smart geometric design is a key enabler of efficient mixing process in this type of micromixers. They can be manufactured in a simple way, using low-cost fabrication techniques and can be easily integrated with other functional components in Lab-on-chip [12] or Organ-on-chip [13] concepts, which are very popular nowadays. Taking into account above-mentioned advantages, this paper analyses fabrication process and testing of performances of a passive micromixer with innovative design. Various technologies can be used for manufacturing passive micromixers such as: printed circuit board (PCB) [14], low temperature co-fired ceramics (LTCC) [15], xurographic PVC foils-based technique [16], silicon-based or glass-based microfabrication [17], polydimethylsiloxane (PDMS) technology [18]. An integrated microfluidic chip for rapid mixing of multiple liquids which requires few manipulations of pipettes has been developed in [19]. That microfluidic chip was fabricated by PDMS casting, integrated a micromixer among four other components. The lost-wax casting technique was used in [20] for fabrication of 3D PDMS microfluidic devices, but with very simple design. The fabrication of PDMS microfilters, which were chemically bonded to polyimide (PI), polyethylene-naphthalate (PEN), and polyethylene-terephthalate (PET) substrates, was demonstrated in [21]. With regard to different geometrical shape of microchannels as a main constituent of passive micromixers, the following structures have been reported: lamination mixer [22], rotational-type mixer or spiral microchannels [23], groove micromixer [24], staggered herringbone mixer [25] and splitting and recombining the flow mechanism [26].

Manufacturing of high-performance microfluidic devices can be challenging, in particular when utilising complex structures of versatile and inconsistent shapes. This study investigates innovative and complex designs of micromixer and their fabrication using PDMS-based platforms. PDMS is chosen for the fabrication of the micromixer due to its biocompatibility, transparency and mechanical flexibility. Design is based on semi-circular shapes of barriers creating disturbances aiming to achieve better mixing performances. The experimental testing of the fabricated microfluidic chip has been also performed.

2 Design, simulation and fabrication of micromixer

2.1 Design of micromixer

The proposed innovative design of the microfluidic micromixer, including its dimensions is presented in Figure 1. The chip is composed of two inlets and one outlet. Internal structure of the chip consists of semi-circular barriers which could provide the chaotic advection with diffusion and enable efficient mixing of fluids from inlets. The calculation of the Reynolds number in the mixing area of 2000 µm width and 60 µm depth was between 0.2 - 3.0, therefore the chaotic advection with diffusion takes place. A design of microchannels was created in CleWin 4.0 software tool.

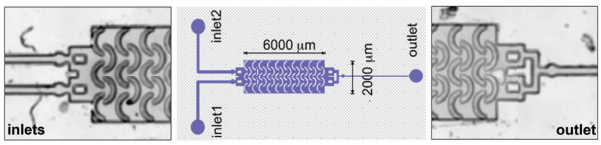

Figure 1: Design and dimensions of microfluidic chip showing the positions of the inlets, the mixing chamber and the outlet

2.2 Simulation results for the proposed micromixer

The proposed micromixer performances have been analysed through simulation results. Numerical simulation was carried out by software tool COMSOL multiphysics, using two modules Laminar Flow and Transport of Diluted Species. The Laminar Flow module has been used for determination of the speed field vector which then transferred into the Transport of Diluted Species module which calculates the concentration of fluid in the designed micromixer. In order to provide

evidence that proposed mixer operates properly we started simulation with the following parameters: the fluid pressure at inlets equal to 50 mbar, concentration of the red fluid (on one inlet) was 500 mol/m^3, and on another inlet (blue fluid) 0 mol/m^3. The obtained results of simulation are presented in Figure 2.

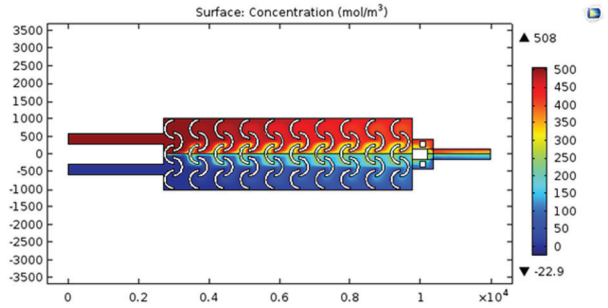

Figure 2: Simulation results of the micromixer design

From the presented simulation results it can be concluded that around internal semi-circular barriers good mixing of two fluids from inlets is performed.

2.3 Fabrication of micromixer

There are two main steps to manufacture a microfluidic chip in PDMS technology: (1) fabrication of a mould and (2) replica moulding. To fabricate a mould, photolithography was used to form a pattern of the designed channels as a moulding master on a glass substrate (75 mm × 50 mm size, 0.96 mm thickness, Corning, USA). A glass substrate was cleaned with isopropanol and heated at 120 °C for 15 minutes. A dry film photoresist (30 µm thickness, ORDYL) was covered and laminated on the glass substrate using a laminator. Using cutting-edge technologies and in-house built in systems, the first protective film was removed and then the resist was exposed for 10 seconds with an UV light (UV-KUB2) using the photomask. After exposing, the second protective film was removed. To etch the pattern, the resist was developed with agitated manually for 2 minutes in a bath containing commercial developers. Then it was rinsed with the commercial chemical, following with isopropanol. Finally, the mould was dried with compressed air. The process of mould fabrication is shown in Figure 3.

Once the mould was created, the microchannels were built using chemically integrated polymer-copolymer matrices, namely polydimethylsiloxane (PDMS), by replica moulding. Using soft lithography, PDMS was used to replicate the patterned glass substrate served as a master. A mixture of PDMS base and curing agent (Sylgard 184, Dow Corning, USA) was prepared in the ratio of 10:1 by weight, and was stirred vigorously until well mixed. The mixture was degassed using a vacuum

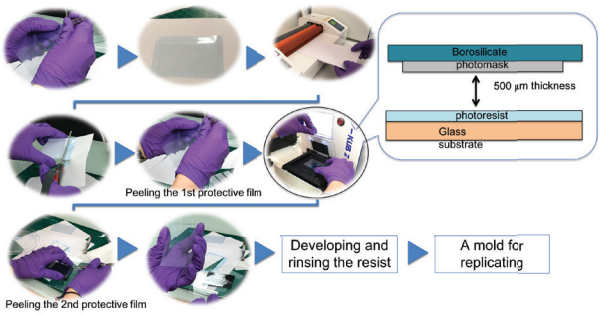

Figure 3: The process of mould fabrication using photolithography process

chamber in order to eliminate air bubbles for 30 minutes. Next, the mixture was cast onto the master. The master filled with PDMS was cured in an oven at 80 °C for 2 hours. Then the replica was cut along the edge of the mould using a scalpel blade and was peeled off from the master. To access holes for the fluidic inlets and outlet insertion point, the PDMS chip were punched using a biopsy punch (1 mm in diameter, Tedpilla). The surface of the punched PDMS chip was exposed to oxygen plasma for 2 minutes, and was placed as soon as possible against a clean glass slide to form a permanent bond. The process of PDMS replication is illustrated in Figure 4.

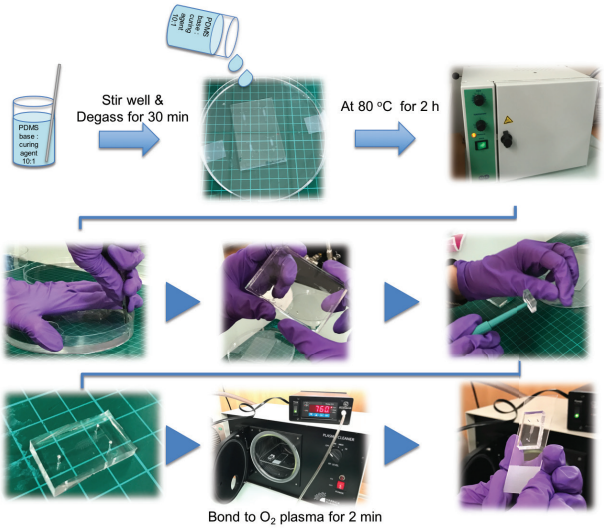

Figure 4: The process of PDMS replication using soft lithography

3 Experimental method

After microfluidic chips were fabricated, the pattern of microfluidic chip was examined under microscope. The size of microfluidic chip was comparable to one-euro coin as shown in Figure 5A. The measurements were

performed in a mixing channel with a cross-section of 2 × 6 mm^2. The first part of the channel was divided into two small inlets, and the shape of semi-circular was used as barriers in the mixing channel (the middle part) as shown in Figure 5B.

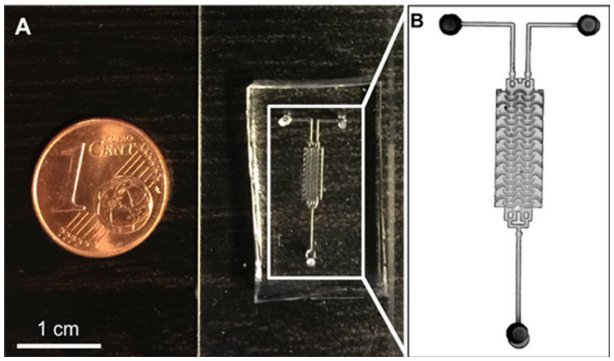

Figure 5: (A) Prototype of the fabricated microfluidic chip, (B) Microscope images showing the pattern inside microfluidic chip

The goal of microfluidic mixing is a thorough mixing of two or more samples in microfluidic devices. In order to achieve that goal, microchannel was designed with barriers to create the chaotic advection with diffusion (Reynolds < 10). Herein, the mixing pattern of the proposed microfluidic chip was studied with microfluidic flow controller (OB1) from Elveflow®. The experimental set-up of microfluidic system is shown in Figure 6.

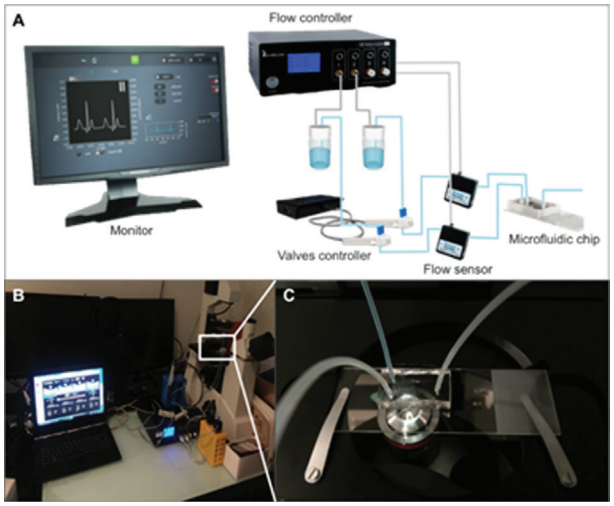

Figure 6: (A) Schematic illustration of experimental set-up for microfluidic mixing study. (B) Photo of experimental setup. (C) Zoom-in version of microfluidic chip under microscope

The flow controller was connected with vacuum pump, which is the pressure supply to the system. Pressure or flow was monitored by the Elveflow Smart Interface on computer. Microfluidic valves controllers were used to quickly start/stop flow. Flow/pressure sensor was also connected to the system for automatically adjusting the pressure in order to reach the set flow rate value. With this set-up, the pressured liquids from samples were smoothly and precisely flowed into microfluidic chip at desired rate. Flow in the chip was then observed under microscope.

4 Results and discussion

4.1 Testing of mixing performances at various pressures

In this study, liquid pressure was varied from 40-200 mbar. The pressure for each channel was set at desired value. Two channels were set at same pressure flows. One channel represented the drug, which was in blue solution and another channel contained just water, which represented normal saline solution using to dilute the drug. At the first stage, the accuracy of the system was verified. At predetermined pressure, we collected samples at each pressure for 6 minutes. The representative of samples collected in each set of experiment is shown in Figure 7A. The measured volumes and appropriate flow-rates are presented in Figure 7B.

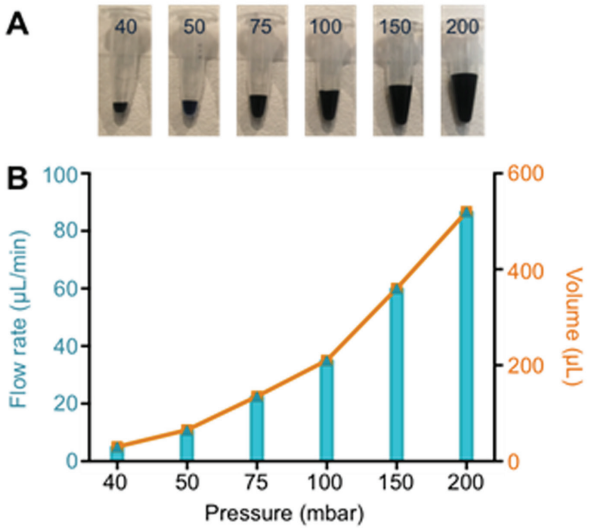

Figure 7: (A) Representative photos of samples collected at various pressure flows. (B) Volume and flow rate at studied pressure range

The flow-rate results were consistent with controlled pressured as expected. The obtain results indicate that the flow was precisely tuned and controlled as it was desired in this experiment. This precise flow control is important for flow transition into microchannel in order to simulate controlled drug injection into the microfluidic system.

4.2 Evaluation of mixing efficiency

To investigate the mixing performance, the standard deviation of the concentration of the colouring solution was calculated by a custom JavaScript in ImageJ. Briefly, the captured images were split into 8-bit images of red, green, and blue components. The red component was only used for measuring the intensities pixel-by-pixel via the detection zone. A standard curve relating intensities and concentrations was established. After converting the intensities to the concentrations, the homogeneity was represented by the standard deviation of the concentration over the detection area. The standard deviation can be normalized by the mean concentration to calculate the mixing index (MI): that was defined as in Eq. (1):

$$MI = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\frac{\left(C_i - \overline{C}\right)^2}{\overline{C}}} \quad (1)$$

Where Ci is the concentration of pixel i, N is the number of pixel over detection zone, and $\overline{C}$ is the ideally average concentration at well-mixing which is equal to 50% of the initial concentration. The standard deviation will be 0 for well-mixing. The mixing efficiency was defined as in Eq. (2):

$$\eta = 1 - \mathrm{MI} = 1 - \sqrt{\frac{1}{N}\sum_{i=1}^{N}\frac{\left(C_i - \overline{C}\right)^2}{\overline{C}}} \quad (2)$$

The efficiency is therefore between $0 < \eta < 1$. When $\eta = 0$ means the highest variation of concentration, *i.e.* no mixing. While $\eta = 1$ is well-mixing.

To relate the intensity with the corresponding concentration, the standard curve was evaluated. The preparation was used the concentration of 10 % (V/V) liquid food dye red in water solution. To cover the concentration, range from 0 – 100%, the standard curve was fit by a polynomial.

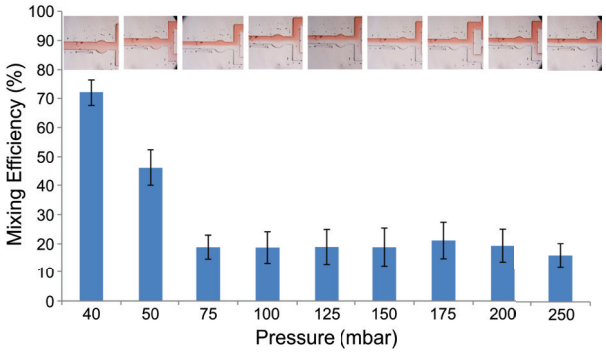

Figure 8: Mixing efficiency and enlarged images of outlet are representatives of the fluid flow within micromixer at different pressure flows

In addition, the mixing was investigated with different pressure set-ups. The liquid 1 (red colour) and liquid 2 (water), at room temperature, entered the inlets from separated channels. The flow in the proposed microfluidic chip was observed under microscope. The mixing efficiency was performed and mixing was quantified using above-mentioned equations. The results are illustrated in Figure 8. The mixing efficiency of is 72% at 40 mbar. It can be observed that at lower pressure (≤50 mbar), two solutions were mixed thoroughly inside microfluidic chip as shown in Figure 9. The proposed micromixer efficiency around 72 % for low pressure, is comparable with the already reported mixing efficiency. For example, in paper [27], authors reported the following maximum efficiency 69, 75, and 79 % for three types of configurations of meander types of microchannels. Additionally, in the paper [28], authors presented T-micromixer's with efficiency around ~20% at similar pressures.

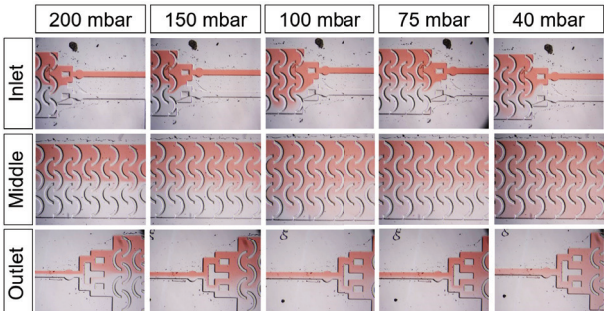

Figure 9: Microscopic images (4×) obtained by mixing experiment in micro-mixer device at various pressure flows

At high-pressure flow (>75 mbar), the mixing did not occur efficiently, because the high velocity flow rate along channel was generated, resulting in fluid could not travel across the channel by the chaotic advection at a very short period of time. Even though, the mixing was not efficient at high flow rates, the diffusion area was larger when flow rate was slower. However, design of a passive micromixer is dictated by its intended application. For example, for chemical reactors and for biosensing application, the flow rate and applied pressure of the tested liquid should be small. That means, the mixing effect in the microfluidic chip should be performed by diffusion. We proposed semicircular barriers in one row 3 and in the next row 2, shifted in the space. In this way, it is achieved that fluid going between these barriers and diffusion effect are pronounced and chaotic mixing realized. Having one and the next rows close to each other, the good mixing efficiency can be reached only for low flow and small

pressures, which is appropriate for intended (above-mentioned) applications of the proposed microfluidic chip.

5 Conclusion

Findings of this study showed that the new design of micromixers exhibits preferable features with promising efficacy in biomedical and fluid-mixing applications. The use of PDMS has enhanced the performance of the micromixers and enables improved flow-mixing properties, which has significant implications in biomedical sensors and molecular-based detection capabilities. The ability of the newly designed micromixers to mix fluids of solution at a significantly low pressure (<50 mbar) is indicative of its potential biomedical applications, and thus, future work will aim to enable further development and testing of the device in biological setting such as *in vivo* analyses.

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7 References

1. Yong Hwang S, Jae Seo I, Yong Lee S, Ahn Y. Microfluidic multiplex biochip based on a point-of-care electrochemical detection system for matrix metalloproteinases. Journal of Electroanalytical Chemistry 2015, doi: 10.1016/j.jelechem.2015.08.015.
2. Huang Y, Cai D, Chen P. Micro- and Nanotechnologies for Study of Cell Secretion. Analytical Chemistry 2011; 83: 4393-40.
3. Zhan Z, Yao P, Dong Z, Tung S, Hohnbaum J, Srinivasan B, Li JW. Insulin Detection Based on a PDMS Microfluidic System. Proceedings of the 2010 IEEE International Conference on Nano/Molecular Medicine and Engineering December 5-9, 2010, Hong Kong.
4. Sundra S, Fhong Soon C, Zainal N, Sek Tee K, Ahmad N, Hua Gan S. The Effects of Micromixing Two Solutions of Two Concentrations in a Two Tier PDMS Micromixer. IOP Conf. Series: Materials Science and Engineering 2017; 226: 012121 doi:10.1088/1757-899X/226/1/012121.
5. Lee CY, Chang CL, Wang YN, Fu LM. Microfluidic mixing: a review. Int J Mol Sci. 2011; 12: 3263-3287.
6. Oddy MH, Santiago JG, Mikkelsen JC. Electrokinetic instability micromixing. Anal. Chem. 2001; 73(24): 5822-5832.
7. Scott Lynn N, Henry CS, Dandy DS. Microfluidic mixing via transverse electrokinetic effects in a planar microchannel. Microfluid Nanofluid. 2008; 5: 493-505.
8. Yang Z, Matsumoto S, Goto H, Matsumoto M, Maeda R. Ultrasonic micromixer for microfluidic systems. Sensors Actuators A Phys. 2001; 93(3): 266-272.
9. Wen CY, Yeh CP, Tsai CH, Fu LM. Rapid magnetic microfluidic mixer utilizing AC electromagnetic field. Electrophoresis. 2009; 30(24): 4179-4186.
10. Liu F, Zhang J, Alici G, Yan S, Mutlu R, Li W, Yan T. An inverted micro-mixer based on a magnetically-actuated cilium made of Fe doped PDMS. Smart Mater. Struct. 2016; 25: 095049 (7pp).
11. Stroock AD, Dertinger SK, Ajdari A, Mezić I, Stone HA, Whitesides GM. Chaotic mixer for microchannels. Science. 2002; 295(5555): 647-651.
12. Schumacher S, Nestler J, Otto T, Wegener M, Ehrentreich-Förster E, Michel D, Wunderlich K, Palzer S, Sohn K, Weber A, et al. Highly integrated lab-on-chip system for point-of-care multiparameter analysis. Lab on a Chip 2012; 12(3): 464-473.
13. Balijepalli A, Sivaramakrishan V. Organs-on-chips: research and commercial perspectives. Drug Discovery Today 2017; 22(2): 397-403.
14. Cabello M, Aracil C, Perdigones F, Quero JM. Conditioning Lab on PCB to Control Temperature and Mix Fluids at the Microscale for Biomedical Applications. Spanish Conference on Electron Devices (CDE), 2017.
15. Malecha K, Golonka LJ, Baldyga J, Jasinska M, Sobieszuk P. Serpentine microfluidic mixer made in LTCC. Sensors Actuators B Chem. 2009; 143(1): 400-413.
16. Chirasatitsin S, Kojić S, Stojanović G. Optimization of microchannel fabrication using xurographic technique for microfluidic chips. Scientific conference ETIKUM'2017, Novi Sad, Serbia, December 06-08, 2017.
17. Silverio V, Cardoso S, Gaspar J, Freitas P, Moreira ALN. Design, fabrication and test of an integrated multi-microchannel heatsink for electronics cooling. Sensors and Actuators A: Physical. 2015; 235: 14-27.
18. He W, Xiao J, Zhang Z, Zhang W, Cao Y, He R, Chen Y. One-step electroplating 3D template with gradient height to enhance micromixing in micro-

fluidic chips. Microfluid Nanofluid, DOI 10.1007/s10404-015-1607-z.
19. Chen PC, Chen YC, Tsai CM. Microfluidic chip for rapid mixing and uniform distribution of multiple reagents using commercial pipettes. Microelectronic Engineering 2016; 150: 57-63.
20. Chung C, Chen YJ, Chen PC, Chen CY. Fabrication of PDMS Passive Micromixer by Lost-Wax Casting. International Journal of Precision Engineering and Manufacturing. 2015; 16(9): 2033-2039.
21. Lee M, Lopez-Martinez MJ, Baraket A, Zine N, Esteve J, Plaza JA, Ahmed N, Elaissari A, Jaffrezic-Renault N, Errachid A. Combination of PDMS microfilters and micromixers based on flexible thermoplastic films for size sorting and mixing of microparticles. J. Appl. Polym. Sci., 2015; 42088 (1 of 12), DOI: 10.1002/APP.42088.
22. Cha L, Kim J, Ryu SK, Park J, Jeong Y, Park S, Park S, Kim HC, Chun K. A highly efficient 3D micromixer using soft PDMS bonding. J Micromech Microeng. 2016; 16:1778-1782.
23. Sudarsan AP, Ugaz VM. Fluid mixing in planar spiral microchannels. Lab Chip. 2006; 6:74-82.
24. Stroock AD, Dertinger SK, Whitesides GM, Ajdari A. Patterning flows using grooved surfaces. Anal Chem. 2002; 74:5306-5312.
25. Stroock AD, Dertinger SKW, Ajdari A, Mezic I, Stone HA, Whitesides GM. Chaotic mixer for microchannels. Science 2002; 295:647-651.
26. Abadi HS, Packirisamy M, Wuthrich R. High performance cascaded PDMS micromixer based on split-and-recombination flows for lab-on-a-chip applications. RSC Advances 2013; 3:7296.
27. Kuo JN, Jiang LR. Design optimization of micromixer with square-wave microchannel on compact disk microfluidic platform. Microsyst Technol, 2014; 20:91-99.
28. Nimafar M, Viktorov V, Martinelli M. Experimental investigation of split and recombination micromixer in confront with basic T-and O-type micromixers. International Journal of Mechanics and Applications. 2012;2(5):61-9.

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