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The Experimental Study of Lab-on-a-Chips for Sheep Blood

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Abstract

This study focused on two types of lab-on-a-chips (LOCs). The first type called the reusable Polydimethylsiloxane (PDMS) LOC was studied to pivote on problems in fabricating it from the literature and improving its performance; less leakage and less analysis time. The reusable PDMS LOC was casted by using the silicon wafer mold. This experimental study also concentrated on fabricating a new PDMS LOC which was casted on a stainless steel mold imported from Bilkent University, Turkey. The stainless steel mold was sketched by CAD program and machined by high-precision CNC; the stainless steel mold is absolutely different from the silicon wafer one. The leakage problem of the reusable LOC which was sandwiched by H-acrylic plates was solved by sticking a tape on PDMS. The hydrophobic behavior of the channels in both LOCs were solved by flowing the bovine serum albumin (BSA) solution before the biological sample was injected. From results obtained by applying the biological sample, sheep blood, on the reusable LOC and adjusting the electrical voltages from 1 volt to 10 volts at 200 Hz constant electrical frequency, we found that the red blood cells (RBCs) moved to the positive electrode at 2 volts, the RBCs started gathering at 3 volts, the RBCs flowed slowly and viscously from 5 volts to 9 volts and, at 10 volts, the sample stopped flowing because the RBCs were dried. From results obtained by applying the biological sample on the new LOC and adjusting the electrical voltages at 0 volt, 1 volt and 5 volt, we found that the sample flow without the electrical potential (0 volt) was slower than the flow with the 1-volt electrical potential but the flow was stopped when the electrical potential at 5 volts was applied. So the new LOC could prove to be used with the sheep blood with the 1-volt electrical potential. From this project, the new LOC showed its potential to be developed continuously with the help of Nanoelectronics and MEMS Laboratory, Thailand National Electronics and Computer Technology Center, and Microfluidics & Lab-on-a-chip Research Group, Bilkent University.

Keywords: PDMS LOC, Sheep Blood, Lab-on-a-Chip.

1. Introduction

Lab-on-a-chip or LOC devices are miniature laboratories built on a thin glass or plastic chip of several centimeters in dimensions. The polydimethylsiloxane (PDMS) LOC is one of the simple LOC devices [1,2]. The PDMS LOC chamber is made from polydimethylsiloxane by solven casting and drilling. These small devices can duplicate the specialized functions as their room-sized counterparts in clinical diagnoses. The advantages of these devices include significantly reduced reagent consumption, short analysis time, automation, and portability [1,2]. There have been several applications using electrokinetic effects for manipulating microparticles, they showed that they were efficient for the biological and medical applications [1–8]. Dielectrophoresis (DEP) is an electrokinetic movement of the neutral particles induced by polarization in non-uniform electric field [2,9]. When the dielectrophoresis is positive, the particle moves towards the locations with the greatest electric field. On the other hand, if the dielectrophoresis is negative and the particle is pushed from the locations with the greatest electric field [2,10,11]. The electrodes of the LOC are normally used to produce an electric field and the DEP. Since the strength of the dielectric force depends strongly on the medium, the particle electrical properties, the particle shape and size, the thickness of the electrodes and the frequency of the electric field, varying these parameters can affect the particle manipulations in many applications. Since the electrode thickness and properties can increase the strength of the dielectric force, increasing electrode thickness and varying electrode materials should be considered when the DEP plays an important role [2].

Li and Cetin [12] introduced a LOC design of a microfluidic channel with a pair of simple electrodes to perform a continuous separation of particles/cells based on their electrical properties using alternating current dielectrophoresis (AC-DEP). They showed their simulated results which were the separation of the spherical particles and the feasibility of the design.

They emphasized that the mean velocity inside the separation channel and the applied voltage across the separation channel effected on the performance of the LOC device.

Cetin et al. [13] presented a novel, simple LOC device for continuous separation of particles by their sizes based on AC-DEP. They applied the non-uniform electrical field generated by means of embedded, 3-D, asymmetric electrodes inside the LOC device. Their electrodes were manufactured by a simple and inexpensive technique extended from the soft-lithographic fabrication method. Their device was applied by low AC electrical potential and provided the successful separations of 10 and 5 mm diameter latex particle mixture and mixture of yeast cells and white blood cells (WBCs). Later, Cetin and Li [14] presented the LOC device, in Fig. 1, which can perform a continuous separation of particles and cells based on their electrical properties using AC-DEP as the proof-of-concept experiments, the manipulation of the particles with n-DEP (latex) and p-DEP (WBCs) response was analyzed by using a design with two inlet reservoirs.

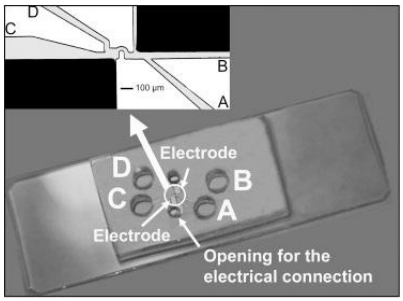

Fig. 1 The final device from Cetin and Li [14]

The review about DEP in microfluidics technology was published by Cetin and Li [15]; they summarized that DEP could be utilized either by DC-field or AC-field and concluded possible future research directions on DEP research as (i) replacing the bench-top instruments with the microfluidics technology for clinical application, (ii) developing the microfluidics technology to hand-held, point-of-care testing devices (both the sample preparation and the chemical/biological analysis in one device), (iii) utilizing mechanical micromachining (milling, drilling), microinjection molding techniques [16,17] for LOC fabrication and using the polymer-based conductive materials as electrodes which may lead to inexpensive and massive fabrication of DEP-based microfluidic systems, (iv) integrating the electrorotation analysis with DEP-based systems would outcome robust and practical DEP based clinical instruments, (v) extending the proposed systems to operate with high-conductivity buffer solutions and (vi) manipulating CNTs and nanoparticles in the LOC devices for the development of the bionano/nanotechnology-based devices and nanomaterial-based sensors.

Cetin and Zeinali [18] presented a numerical modeling using COMSOL Multiphysics for the separation and counting of (bio)-particles by applying 3D sidewall electrodes over planar electrodes due to the use of low voltage and having the same DEP force throughout the channel height. Later, Zeinali et al. [19] introduced their pioneer study in the fabrication of microfluidic devices with embedded 3D electrodes and PZT slides as shown in Fig. 2. This new design was also aimed to separate particle/cell mixtures as presented by Kang et al. [20]. Zeinali et al. [19] utilized the mechanical (CNC-based) machining provided limitations for high resolution and complex structures but some their unique advantages over the lithography-based fabrication were long lasting and durable molds, no need for high facility cleanrooms, ease of controllability due to fewer control parameters and ease of fabrication of high aspect ratio channels for high throughput microfluidic device.

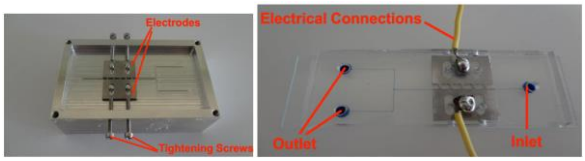

Fig. 2 Mold and the assembled DEP device with 3D sidewall electrodes proposed by Zeinali et al. [19].

The LOCs have been developed continuously in Thailand and there are some LOC applications that are necessary for local utilizations such as the swine and avian flu detecting applications. Some researchers developed the LOC virus detectors [1] since the World has been threaten by the avian influenza and H1N1 pandemic [2]. Pramuanjaroenkij et al. [2] presented the reusable Lab-on-a-Chip or LOC was fabricated and developed to reduce its investment cost and to ease its operations by applying the LOC introduced by Cetin et al. [13]. In this work, chicken and sheep blood were chosen as the samples to implement two-phase flows with different red-blood-cell shapes. The chicken blood flow represented the viscous two-phase flow with the oval shape particles while the sheep blood flow represented the inadhesive samples with the round particles. Their LOC electrodes from different materials as the sputtered nickel plate, the aluminium foil, the copper plate, and the gold foil were examined. Ratios between the anticoagulant solution and the biological samples were studied to find their effects on sample velocities and to find the best image to characterize the RBC flow behaviors. Since it was hard to characterize the particle flow in the inadhesive flows, the result pictures were analyzed and presented in terms of color intensities per unit area by using a computer program called "ImageJ". The sheep-blood-flow results were validated with the hematology results which were the hematocrits to find their relationships between the hematocrits and the RBC flows. Among different LOC electrodes, the sputtered nickel electrodes were the most suitable electrodes in this current application. We found

that the suitable anticoagulant-sample ratios for chicken and sheep samples were 5:1 and 1:1 by volume, respectively. The normal-health-condition sheep with the standard hematocrits higher than 28% showed the average-different-color intensities per unit area between cathode and anode at 39.485 pixels per unit area while the lower standard hematocrit samples, the hematocrits were lower than 28%, showed the average-different-color intensities at 14.641 pixels per unit area, the lower intensity the lower hematocrit. So the LOC coupled with "ImageJ" exhibited their capabilities to investigate the sheep blood conditions, especially, this coupled technique consumed less time than the traditional hematology process [2].

In the current work, sheep blood was chosen to be the biological samples to be investigated. Two types of lab-on-a-chips (LOCs) were studied; the reusable PDMS LOC [2] introduced by Cetin and Li [14] and a new PDMS LOC casted on the stainless steel mold proposed by Zeinali et al. [19]. The reusable PDMS LOC was investigated to solve its fabrication problems and improve its performance; less leakage and less analysis time. To find the proper electrical field where the biological samples were not damaged in the reusable PDMS LOC, the electrical potential effects on the sample flow with different current voltages in the reusable LOC were investigated. The new LOC casted on the stainless mold was brought to Thailand and it was applied with livestock biological samples to find its feasibility to be developed in any livestock researches as in the literature [2] because we did not have high cost facility as cleanrooms and we could fabricate the stainless mold by utilizing the CNC-based machining with high aspect ratio channels for high throughput microfluidic device. Therefore, this work was aimed to improve the performance of the reusable PDMS LOC, to find the effects of the electrical potential inside the reusable PDMS LOC on the sheep blood, and, finally, to evaluate the feasibility in using the PDMS LOC casted on the stainless steel mold with the sheep blood.

2. Experimental Study

The silicon wafer was used to produce a master prototyping of the PDMS microstructure and was patterned by using the negative photo-resist (SU-8 25, MicroChem Co., Newton, MA) technique. The dielectrophoretic chamber was made from the PDMS prepared by mixing the precursors sylgard with a curing agent at a ratio of 10:1 by volume. The prepolymer mixture was degassed at 20-50 mTorr at room temperature in desiccators pumped with a mechanical vacuum pump for 10 minutes to remove any air bubbles in the mixture. The PDMS mixtures were gradually poured onto the patterned silicon wafer or a mold. After the PDMS was cured at 100^{o}C for 30 minutes on the mold, the molded polymer samples were peeled off and punched into a hole in order to create a chamber. The microelectrodes were inserted into the PDMS electrode chambers manually under the microscope and, then, all components as the LOC were assembled by using acrylic plates to sandwich the components. In each LOC device, there were two inlet reservoirs, two exit reservoirs, channels with 25 micrometers (in z-direction) in height, the main channel with 100 micrometers in width, and two small reservoirs punched on top of the electrodes for the external electrical connections [2,21,22].

Firstly, the electrodes made from nickel by using the sputtering technique performed by the commercial sputtering system were fabricated. Then, the electrodes were placed on the electrode positions on the PDMS casted from the silicon wafer. At the end, the glass slide was placed on top of the first two parts and all three parts were sandwiched by H-acrylic plates called the reusable PDMS LOC.

Secondly, the new PDMS LOC was casted on the stainless steel mold imported from Bilkent University, Turkey., there were total of four screws on both sides of the mold; two screws for each side, to hold the electrodes on their positions, control the PDMS flow and to prevent PDMS leakage. The new PDMS LOC was fabricated at Nanoelectronics and MEMS Laboratory, Thailand National Electronics and Computer Technology Center by following steps; (i) washing the stainless steel mold with Isopropanol, (ii) placing the stainless steel electrodes on the stainless steel mold (Fig. 3), (iii) mixing PDMS and water with ratio of 10:1 and pouring the mixture on the stainless steel mold, (iv) placing the stainless steel mold inside the vacuum chamber to get rid of air bubbles, (v) placing the mold on the hotplate with constant temperature, (vi) taking the PDMS attached with the electrodes out of the mold and placing the glass slide on the PDMS and (vii) placing both parts inside the plasma cleaner for 10 minutes, called the new PDMS LOC.

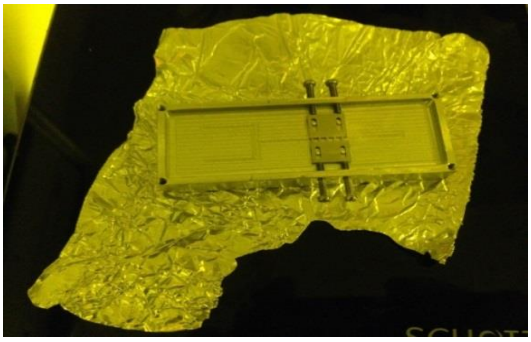

Fig. 3 The stainless steel electrodes on their positions on the stainless steel mold [23].

Finally, the sheep blood was tested inside both LOCs. The reusable PDMS LOC was primarily investigated to improve its fabrication problem which was its leakage by adjusting the reusable LOC before sandwiching it with the H-acrylic plates. The reusable LOC was improved its performance by solving its hydrophobic behavior with the bovine serum albumin (BSA) solution. Then, the electrical potential effects on the sample flow were investigated in this reusable PDMS LOC by altering the electrical potential of the

electric current fed to the electrodes from 1 volt to 10 volts. Furthermore, the new PDMS LOC which was casted on the stainless steel mold was investigated with the sheep blood to find the flow patterns to show its potential to be used with the sheep blood.

3. Results And Discussion

The sheep blood was chosen as the biological sample, taken from the sheep by the professional caretaker (Fig. 4(a)) and prepared by mixing the blood with the ethylenediaminetetraacetic acid (EDTA) as the anticoagulant solution, we already found that the suitable anticoagulant-sample ratio for the sheep sample at 1:1 by volume from our previous work [2]. The biological sample was put in separated tubes to be ready for the test as shown in Fig. 4 (b).

(a)

(b)

Fig. 4 The sheep blood withdrawn by the professional caretaker [23] and ready to be tested.

The nickel electrodes were placed on the electrode positions on the PDMS casted from the silicon wafer and then the glass slide was placed on top of the first two parts, we applied the sticky tape to attach all three parts to improve its leakage problem. Then the reusable LOC was sandwiched by H-acrylic plates and connected with the wires to be ready for the biological sample test as shown in Fig. 5. To check its leakage, the BSA solution was fed into the reusable LOC, we found that the solution could flow without any leakages. We repeated applying the sticky tape to attach all three parts, feeding the BSA solution and found no leak. To check the advantage of the BSA solution over the channel hydrophobic problem, the reusable LOC was fed with the biological sample, we found that the sample could flow faster than in the literature [2] which the PDMS plate was submerged under water to solve the channel hydrophobic problem, Fig. 6 showed the solution and sample flow inside the channel with no leak.

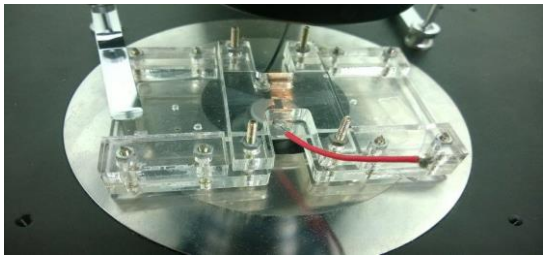

Fig. 5 The reusable PDMS LOC setup for the biological sample test [23].

(a) (b)

Fig. 6 The BSA solution (a) and sample (b) flow inside the channel with no leak.

The electrical potential effects on the sample flow were investigated in the reusable PDMS LOC by altering the electrical potential of the electric current fed to the electrodes from 1 volt to 10 volts at constant frequency of 200 Hz. We measured the time when the sample flow passed the electrode position and plotted the time versus the electrical potential applied to the electrodes as shown in Fig. 7. As one may noticed, the higher voltage or the higher joule heating, the sample flowed slower. We also observed that, at the electrical potential of 2 volts, the RBCs moved forward and closer to the positive electrode than they moved to the negative electrode as shown in Fig. 8. At 3 volts, the RBCs started gathering in some positions on the channel. The sample flow was slower when the electrical potentials were increased and the sample flow was stopped at 10 volts (Fig. 9).

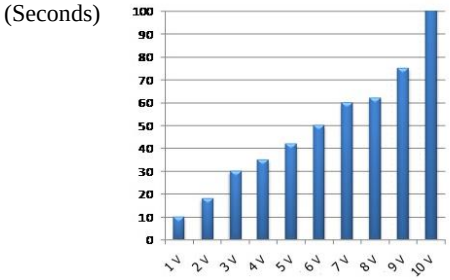

Fig. 7 The sample flow times for different electrical potential [23].

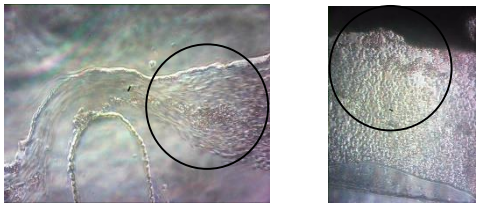

Fig. 8 The sample flow closer to the positive electrode at 2 volts.

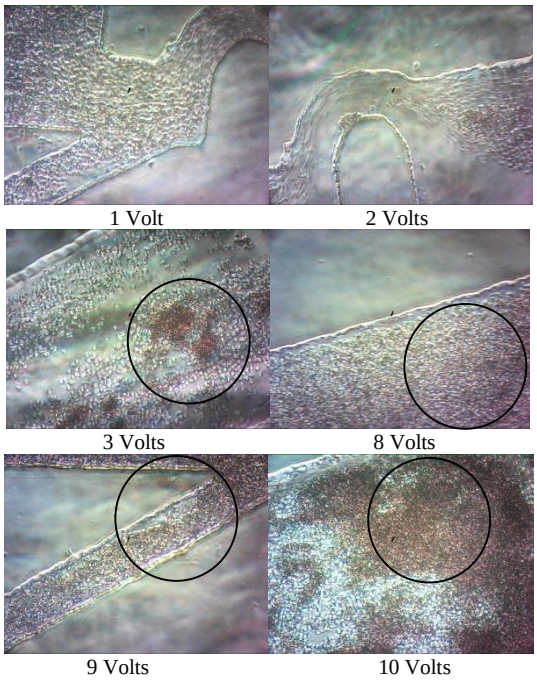

Fig. 9 The sample flow inside the reusable LOC for different electrical potential [23].

For the new PDMS LOC, after placing all parts inside the plasma cleaner for 10 minutes, the new PDMS LOC was ready to be test with the biological sample as shown in Fig. 10. The channel was observed under the microscope and we found the coarse surfaces on the channel walls (Fig. 11). The electrical potential effects on the sample withdrawn from two sheep were also investigated in the new PDMS LOC by altering the electrical potential of the electric current fed to the electrodes at 0 volt, 1 volt and 5 volts at constant frequency of 200 Hz. To show the new PDMS LOC potential to be used with the sheep blood, we took the samples from two sheep and test on the new PDMS LOC. We also flowed the BSA solution to reduce the hydrophobic behavior of the channel and observed that the BSA flow on the sides or edges of the channels as shown in Fig. 12. From results, we found that the sample flow without the electrical potential (0 volt) was slower than the flow with the 1-volt electrical potential but the flow was stopped when the electrical potential at 5 volts was applied. We observed that the coarse surfaces in the middle of the channel caused the flow moved on the sides or edges of the channels before it could move through the middle. We could prove that the new LOC could be used with the sheep blood with the 1-volt electrical potential.

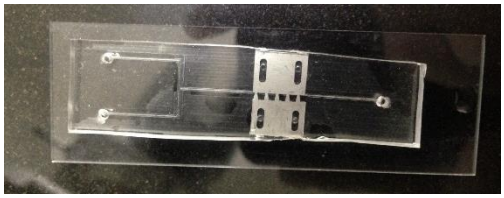

Fig. 10 The new PDMS LOC setup for the biological sample test [23].

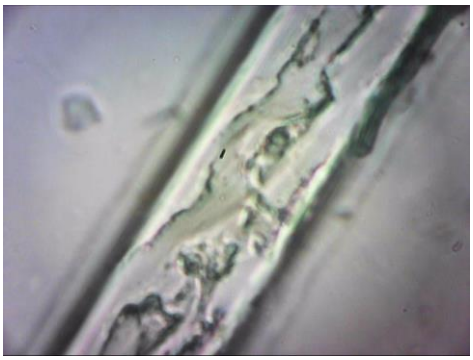

Fig. 11 The coarse surfaces on the channel walls of the new PDMS LOC.

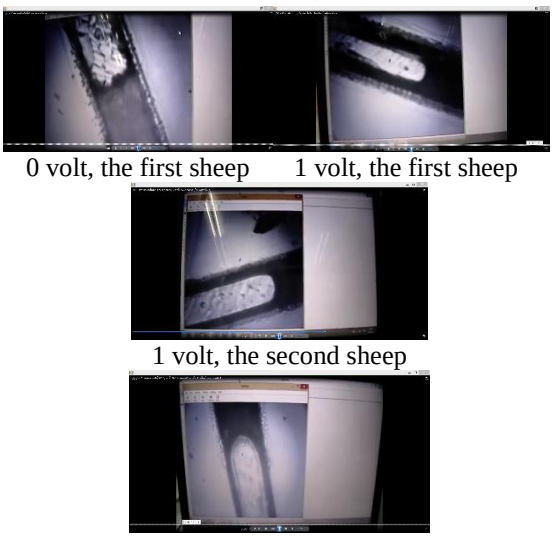

Fig. 12 The coarse surfaces on the channel walls of the new PDMS LOC.

4. Conclusion

The sheep blood was chosen to be the biological samples to be investigated. Two types of lab-on-a-chips (LOCs) were studied; the reusable PDMS LOC [2] introduced by Cetin and Li [14] which was casted by using the silicon wafer mold and the new PDMS LOC casted on the stainless steel mold which was machined by high-precision CNC proposed by Zeinali et al. [19]. The stainless steel mold is absolutely different from the silicon wafer one. The reusable PDMS LOC was studied to improve its fabrication problem and its performance; less leakage and less analysis time. The leakage problem of the reusable LOC which was sandwiched by H-acrylic plates was solved by sticking

a tape on PDMS. The hydrophobic behavior of the channels in both LOCs were solved by flowing the bovine serum albumin (BSA) solution before the biological sample was injected. Additionally, the electrical potential effects on the sample flow were investigated in this reusable PDMS LOC. From results obtained by applying the biological sample, sheep blood, on the reusable LOC and adjusting the electrical voltages from 1 volt to 10 volts at 200 Hz constant electrical frequency, we found that the red blood cells (RBCs) moved to the positive electrode at 2 volts, the RBCs started gathering at 3 volts, the RBCs flowed slowly and viscously from 5 volts to 9 volts and, at 10 volts, the sample stopped flowing because the RBCs were dried.

The new PDMS LOC was fabricated in Thailand successfully and it could be used with the livestock biological samples. From results obtained by applying the biological sample on the new LOC and adjusting the electrical voltages at 0 volt, 1 volt and 5 volt, we found that the sample flow without the electrical potential (0 volt) was slower than the flow with the 1-volt electrical potential but the flow was stopped when the electrical potential at 5 volts was applied. So the new LOC could be proved to be used with the sheep blood with the 1-volt electrical potential. From this work, the new LOC showed its potential to be developed continuously in any livestock researches as in the literature [2] with the help of Nanoelectronics and MEMS Laboratory, Thailand National Electronics and Computer Technology Center, and Microfluidics & Lab-on-a-chip Research Group, Bilkent University. We targeted our aims to fabricate the stainless mold by utilizing the CNC-based machining becaused of its initial mold cost.

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