



Feasibility study on Lab-on-a-chip fabrication in Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus

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Abstract

This study presents problems and results in fabricating microfluidic devices for biological sample manipulation at Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus. Electrodes obtained from sputtering technique are used to produce dielectrophoretic force on the devices. The electrodes were fabricated with three different current conditions. The polydimethylsiloxane (PDMS) lab-on-a-chip (LOC) design from a literature was considered specifically as a microfluidic device prototype. The PDMS LOCs were fabricated manually at Chalermphrakiat Sakon Nakhon Province Campus but all components were prepared and fabricated at Nanoelectronics and MEMS Laboratory, National Electronics and Computer Technology Center. The PDMS LOC was aimed to use with biological samples and different frequencies of AC electric fields. From the LOC fabrication results, the higher current in electroplating process, the coarser electrode surfaces, the finished LOC with these electrodes showed problems in channels. The LOC fabricated locally and manually without using plasma cleaners is reassembly, considered as the main advantage. However, it is required skills and techniques to arrange all components to be on exact positions. This feasibility study shows that there is possibility to develop the LOC technology locally with helps from Nanoelectronics and MEMS Laboratory. The LOCs were also examined with two sets of biological samples, from the results, the different samples reacted differently with the electric field on the workable LOCs.

Keywords: polydimethylsiloxane, PDMS, lab-on-a-chip, LOC

1. Introduction

Lab-on-a-chip or LOC devices are miniature laboratories built on a thin glass (a

glass slide) or plastic chip in centimeter dimensions. These small devices can work 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].

Researches on LOC systems are developed by the aim to miniaturize, integrate, and automate biochemical assays. The development of the LOC systems and devices requires the integration of multiple fluidic functions onto a single system or device with the ability of a well defined fabrication process to fabricate cost efficient and portable devices. Since there is the large diversity in microfluidic developments, collaborations, and supply chains of different companies forming new solutions by just combining off-the-shelf components are still out of reach [2]. Microfluidic platforms provide a variety of design components and fabrication technologies that are well tuned on to each other [3]. The LOC capability enables the development of the LOC systems where complex biochemical assays that commonly require multiple laboratory tasks employing multiple devices are integrated and automatized on a single miniaturized system. In a microfluidic platform, basic fluidic functions (unit operations), are reagent storage, reagent release, fluid transport, fluid metering, fluid mixing, flow controlling, and separation or concentration of molecules or particles [2].

In LOC systems [4], the manipulation of particles and biological cells is crucial in a variety of biomedical applications such as cell separation, cell counting, cell trapping and cell patterning. To achieve these tasks, various techniques have been developed to be used in microsystems such

as optical tweezers [5], magnetophoresis [6], acoustic means [7–9] and dielectrophoresis (DEP). The microfluidic platforms are categorized [2] as lateral flow assays [10], the microfluidic large scale integration approach [11], centrifugal microfluidics [12], pressure driven droplet based microfluidics [13], electrowetting based microfluidics [14], and SAW driven microfluidics [15]. The polydimethylsiloxane (PDMS) LOC is one of the simple LOC devices. LOC chamber is made from polydimethylsiloxane by solven casting and drilling. The polydimethylsiloxane belongs to a group of polymeric organosilicon compounds that are commonly referred to as silicones [16].

2. PDMS LOC technology

The PDMS LOC has been developed continuously in Thailand by limited numbers of research groups. Several LOC applications are imported to Thailand, so their sold prices are high and their configurations depend on their manufacturers. There are some LOC applications necessary for local utilization such as poultry sickness and swine flu detecting application. The researchers developed the LOC virus detectors [1] since the World has been threaten by avian influenza and H1N1 pandemic. Thailand is one of countries threaten by the virus because the agricultural industry is the main industry in the country, so the earlier virus detection is the better solution. Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, is considered as one of leading scientific research institutes and has potential to utilize LOC application since we have agricultural research projects. A research on LOC fabrications will help researchers to



understand all basic factors effecting on basic fluidic unit operations of the LOC systems, help reducing LOC manufacturing cost and help developing special LOC for agricultural applications in Thailand.

2.1 Dielectrophoretic device

Maturos et al. [17] presented techniques used in a LOC system, separation and manipulation of particles using traveling wave dielectrophoretic force. There are several applications using electrokinetic effects for manipulating microparticles, they have been shown to be efficient for biological and medical applications [1,4,18–22]. Dielectrophoresis (DEP) is an electrokinetic movement of neutral particles induced by polarization in non-uniform electric field [23]. The time averaged dielectrophoretic force on a dielectric sphere in non-uniform electric fields is represented by

$$F = 2\pi\epsilon_0\epsilon_m r^3 \left[\text{Re}(f_{CM}) \nabla |E|^2 + \left(-\frac{2\pi}{\lambda} \right) \text{Im}(f_{CM}) E^2 \right] \quad (1)$$

The positive and negative DEP force is determined by the real and imaginary part of a Clausius-Mossotti factor, $\text{Re}(f_{CM})$. When the dielectrophoresis is positive, the particle moves towards the locations with the greatest electric field. Otherwise, if the first term is negative, the dielectrophoresis is negative and the particle is driven out from the locations with the greatest electric field [24,25]. Traveling wave dielectrophoresis (twDEP) is a traveling wave electric field which can be produced when a 90 degree phase shift signal sequence is applied to a parallel electrode array. twDEP occurs when the real part of the force equation is much less

than the imaginary part [26]. Since the strength of the dielectric force depends strongly on the medium, the particle's electrical properties, the particle's shape and size, the electrode thickness and the frequency of the electric field, varying of any parameter allows the manipulation of particles with great selectivity which can be applied in many applications.

2.2 Electrodes

Since the electrode thickness play an important role to increase strength of the dielectric force, increasing electrode thickness should be considered. There are several techniques that can be used to fabricate the electrodes. Two techniques presented here are sputtering and electroplating. Sigmund [27] introduced Sputtering of a target such as ions by energetic ions or recoil atoms is assumed to result from cascades of atomic collisions. There are two main stages of the collision cascade distinguished: first, the slowing down of the primary ion and all recoiling atoms and second, the creation and slowing down of low-energy recoils that constitute the major part of all atoms set in motion. The separation of the cascade into two distinct stages has the consequence that two characteristic depths are important for the qualitative understanding of the sputtering process. Electroplating techniques can be used to increase the electrode thickness after the electrodes were processed by the sputtering technique. The Electroplating technique [28] refers to electrochemical deposition of metals, alloys or semiconductors with direct or pulsating current. In electroplating the current distribution



on the feature scale determines the shape of the growth front in plate-up plating.

2.3 PDMS LOC fabrication

Cetin [4] presented the PDMS microfluidic chip fabrication on a glass substrate following soft lithography protocol. The master for rapid prototyping of the PDMS microstructure was fabricated using negative photo-resist on the glass substrate. In his work, the micro copper electrodes were used. The fabrication procedure of the micro copper electrodes is extended from the soft-lithographic fabrication of the photo-resist master. The microelectrodes are inserted into the PDMS electrode chambers manually under the microscope. The PDMS slab is plasma treated and bonded with the glass substrate to form the microchannel network with embedded electrodes. For Cetin chip, there are two inlet reservoirs, two exit ones, channels with 20 micrometers (in z-direction) in height, the main channel with 200 micrometers in width, and all branches diverging toward the connection section with the reservoirs to increase the rigidity of the chip during the fabrication and two small reservoirs punched on top of the electrodes for the external electrical connections.

In this study, we present a fabrication of twDEP PDMS LOC devices with difference fabrication conditions. The PDMS LOCs were fabricated by using standard microfluidics fabrication techniques coupled with electrode thickened techniques. The effects of difference conditions were observed. The main processes of electrode fabrication consist of sputtering and electroplating. The PDMS LOC from Cetin [4] was

considered specifically in this study as a prototype design because his LOC fabrication is simple; the PDMS LOC fabrication follows processes described in the literature. The workable LOCs is examined with two set of biological samples to observe possibility to use the LOCs with the biological samples.

3. Experimental Study

In this 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 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. PDMS mixtures were gradually poured onto the patterned silicon wafer or a mold. After the PDMS was cured at 100°C for 30 minutes on a mold, the molded polymer samples were peeled off and punched into a hole in order to create a chamber. The mask for fabricating electrode was designed according to Cetin design [4] by using L-Edit software. The chromium electrode array was patterned on glass slides by DC sputtering through microshadow masking. The chromium were sputtering under argon plasma. The sputtering pressure, sputtering current, and time for chromium are 3×10^{-3} mbar, 0.2 A, and 2 minutes, respectively. The sputtering was conducted at room temperature. The chamber



and electrodes were treated under oxygen plasma (Harrick scientific corp. model PDC –32 G) before being attached to each other. The electroplating technique was applied secondly to one set of electrodes by applying electric current at 0.2 Ampere for 1 hour following by applying lower current, 0.07 Ampere. The electrode features were compared between the electrodes produced by only sputtering technique and both techniques.

The microelectrodes are inserted into the PDMS electrode chambers manually under the microscope but there are two ways to assemble the LOCs in this work; one is using plasma cleaner and another one is using acrylic plates to sandwich the components. The completed-workable LOCs were investigated with two sets of biological samples which were prepared from the chickens with poor and good health conditions to see reactions between different sample conditions and the electric field on the LOC.

4. Results and discussion

4.1 Electrodes

Figs. 1 and 2 show the electrode features of the electrodes in LOC devices produced by only sputtering technique and both techniques, respectively. Both LOC devices were assembled by using plasma cleaner. The electrode surface undergone both techniques is coarser, while the electrode surfaces undergone only sputtering technique is smoother, we can notice that the electroplating process effects the electrode surface features. The latter electrodes cannot stick on the glass slides, they peeled out off the slides, and these electrodes can cause problems

in PDMS assembly process. Because if electrode positions are not correct, there are some tolerances, the flow channels are blocked.



Fig. 1 The electrode produced by only sputtering techniques



Fig. 2 The electrodes produced by sputtering and electroplating techniques

4.2 PDMS LOC fabrication

The LOC device in this study consist of 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.

One of problems in LOC devices designed in this study is channel collapse since the channel width is 4 times longer than the height. Fig. 3 shows the enlargement of the circled part in Fig. 1. Since the electrode thickness can increase electric field intensity and the smooth electrodes influence on LOC assembly, the thick electrodes from sputtering technique were performed and assembled with other



components (Fig. 4). As shown in Fig. 4, the PDMS layer does not attach to the glass slide in the area around the thick electrodes and there is channel collapse (indicated by the biggest arrow). Another problem happening in the LOC assembly, the electrodes slightly moved, while we put the LOC assembly inside the plasma cleaner, and overlapped the channel position, so this LOC device cannot work properly.

To study the feasibility of LOC fabrication locally and manually without using plasma cleaners, the first set of LOC devices was fabricated by using plasma cleaner at Nanoelectronics and MEMS Laboratory, National Electronics and Computer Technology Center, Thailand, and another set was fabricated at Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus. The second set is aimed to help solving component alignment problems.

The LOCs were fabricated locally and manually without using plasma cleaners but with using acrylic plates to sandwich the components (Fig. 5). We found that these local LOCs are reassembly, taking apart and assembling repeatedly, while we cannot rearrange any component positions of the LOCs processed with the plasma cleaner. We considered the reassembly as the main advantage. However, it is required skills and techniques to arrange all components to be on exact positions. Since channel collapse is one of LOC fabrication, the channel of the locally assembly LOCs were examined for their flow capabilities. Fig. 6 shows the experimental setup for the study. Fig. 7 shows

the locally assembly LOC with a leaking problem because all components do not attached tightly. Fig. 8 shows the fluid flow inside a channel inside the locally assembly LOC (without a leaking problem). From the results, this feasibility study shows that there is possibility to develop the LOC technology locally with the helps from Nanoelectronics and MEMS Laboratory.



Fig. 3 Channel collapse (enlargement of the circled part in Fig. 1)

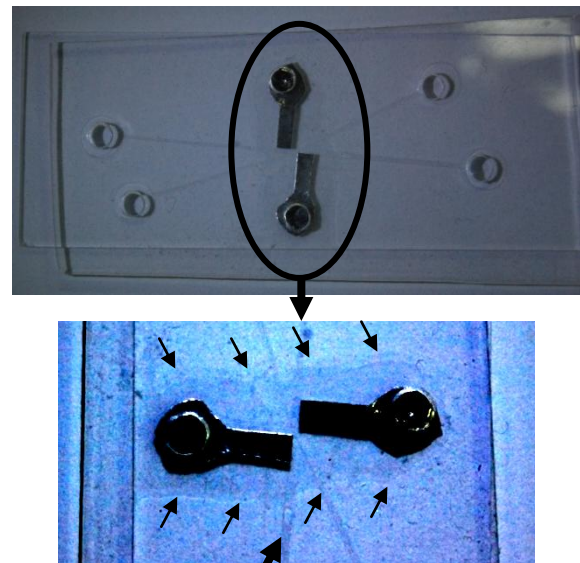


Fig. 4 The LOC with thick electrodes



Fig. 5 The LOC fabricated at Kasetsart University,
Chalermphrakiat Sakon Nakhon Province
Campus



Fig. 6 The LOC experimental setup at Kasetsart
University, Chalermphrakiat Sakon Nakhon
Province Campus

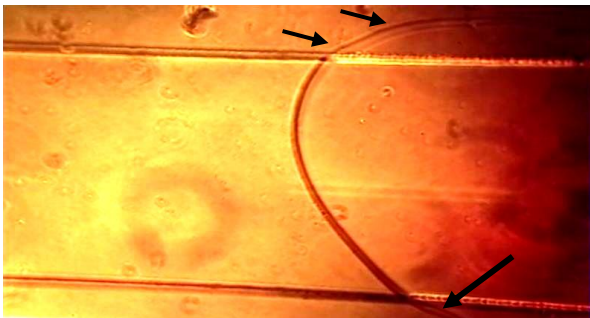


Fig. 7 The locally assembly LOC with a leaking
problem

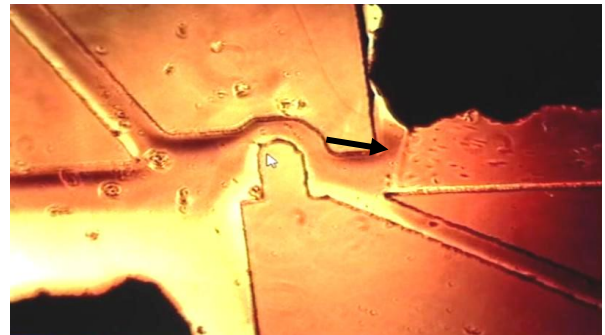
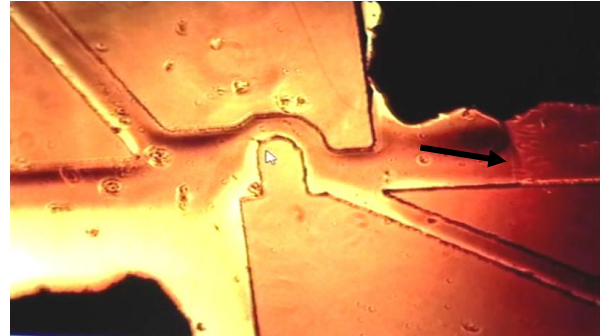


Fig. 8 The locally assembly LOC without a
leaking problem

The last part of our experimental study is to examine the completed-workable LOCs with two sets of biological samples, prepared from chickens with poor and good health conditions. The results showed that red blood cells in the sample from the good health condition can flow and be accelerated pass the electric field (the electrodes) as shown in Fig. 9. But the red blood cells in the sample from the poor health condition cannot flow smoothly pass the electric field (the electrodes) and some of them were moved forward to the electrode as shown in Fig. 10.

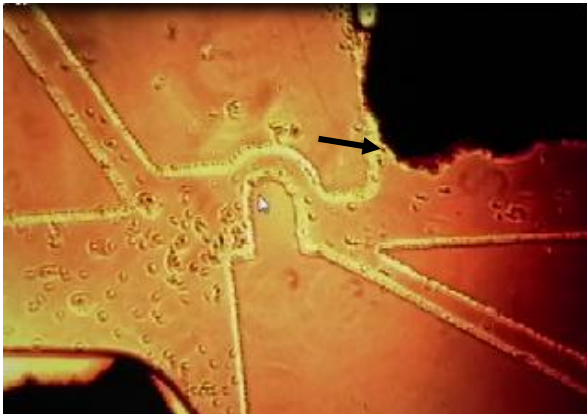


Fig. 9 The workable LOC with the sample from the good health condition.

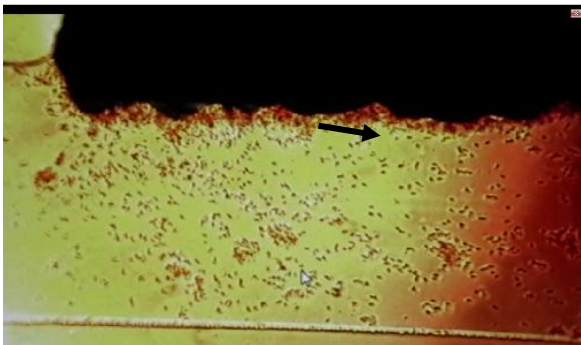


Fig. 10 The workable LOC with the sample from the poor health condition.

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