The 2013 World Congress on
Advances in Nano, Biomechanics, Robotics, and Energy Research (ANBRE13)
Seoul, Korea, August 25-28, 2013

The Investigation to Obtain Relationships between Hematocrit and Flow Patterns on Lab-on-a-Chip

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(Received keep as blank , Revised keep as blank , Accepted keep as blank)

Abstract. From our previous works, Lab-on-a-Chips or LOCs were fabricated and investigated for re-usable designs, the manual LOC with a sandwiched acrylic set was chosen to develop for biological sample investigation. Chicken blood was the biological sample for our primary works. In the current work, the LOC was applied to investigate sheep blood from different sheep conditions, 2 different groups divided by hematocrit conditions. Buffer-sample ratios were also studied to find the suitable ratio. Since the different sheep-blood-flow could not distinguished visibly, result pictures were analysed for an intensity-per-area value of each flow by using a computer program called "imageJ", the program can estimate the intensity-per-area value. The imageJ evaluates the color intensities of red blood cells that move forward to each electrode; anode and cathode. Then the analysed results were validates with hematology results of the sheep to find their relationships. We found that the suitable ratio EDTA and blood was 1:1 by volume because the best image result was obtained. Average different sample-color intensities per unit area from both conditions; standard hematocrit and lower standard hematocrit, were 39.485 and 14.641 pixels per unit area, respectively. The intensities of sample pictures were calculated from subtractions between intensities at anode and those at cathode. So, relationships between hematocrit quantities and the sample-color intensities per unit area at the electrodes showed an initial feasibility to develop a device to diagnose animal symptoms in the future.

Keywords: Hematocrit; Lab-on-a-Chips; Blood; Density-per-area

1. Introduction

Blood is the most important part of human and animals which work to transport necessary substances to cell in every organ, the cells are alive and function properly and efficiently. When every cell works normally, organs and bodies can work accordingly. Blood contains of plasma and corpuscles or formed element. The corpuscles are composed of white blood cells, red blood cells and platelets. So blood conditions can identify health problems in living things. Hematocrit (Hct) or Pack cell volume (PCV) of the red blood cells drawn from patients is a quantity in hematology test to inform their health conditions for medical providers. Hematocrit is evaluated in the volume

percentage (% vol) of red blood cells in blood. So the hematocrit is useful in diagnosis for any disease conditions.

Lab-on-a-chip or LOC devices are miniature laboratories built on a thin glass or plastic chip of several micrometers in dimensions. 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 (Li 2010, Hattori and Yasuda 2012). 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 (Mark 2010). We introduced problems in a manual polydimethylsiloxane (PDMS) LOC assembly by using acrylic plates (Pramuanjaroenkij et al. 2011 and 2012). The manual assembly was chosen as our main fabrication to be developed because it can be developed locally with its effective cost. We already solved some fabrication problems; adhering problems by using an H-acrylic-plate set and hydrophobic problems by using Bovine Serum Abumin (BSA) solution to reduce the PDMS hydrophobic behavior, none damaged cell occurring. The LOC was used to examine samples; chicken blood, obtained from different chicken health conditions and results of the sample flows on the device were different for the different health conditions.

Since LOC configurations which were proper for animal blood samples have been sought, if sample flows on the LOC can be related with animal health conditions, the LOC flow character can also reveal its feasibility to be applied with the animal samples to diagnose the health conditions. First, we solved our manual LOC leaking problems with a new acrylic plate design and tested this manual assembly LOC with biological samples. Next, this work presented relationships between animal hematocrit quantities and sample flow characters on the LOC. Sheep blood was applied in the work; its small red blood cell behaviors were detected and evaluated by using a computer program named “ImageJ”. We also investigated different buffer-sample ratios for their effects on the flow characters and sample coagulation on the LOC.

2. Hematology

Hematology is the study that concerns about blood such as blood counting and can help scientists to diagnose or to treat or, even, to prevent the living things from dangers. One of blood counting methods is the hematocrit. Traditionally, the hematocrit can be determined by centrifuging heparinized blood in a capillary tube at 10,000 revolutions per minute for five minutes (Senagore 2003). The blood was separated into layers; the volume of packed red blood cells divided by the total volume of the blood sample indicated the hematocrit. The packed red blood cell layer in the tube can be measured and compared with the total length; its unit is the volume percentage (% vol) of red blood cells in blood. The hematocrit is useful in diagnosis for any disease conditions such as, in patients with dehydrated conditions, hematocrit in normal people is in a range of 37 – 52%. In another example, patients with anemia, their hematocrit is lower than a standard range. hematocrit in animals is utilized in the same way as in human.

Deviations of animal hematocrits from a normal hematocrit can have important consequences in terms of the ability of blood to carry oxygen. A hematocrit also affects the viscosity of blood, 40% hematocrit blood has twice the viscosity of plasma (acellular or extracellular liquid in blood). When hematocrits are higher than 40%, viscosity increases rapidly. In molecular analysis, standard hematocrits also indicated that the blood contains enough hemoglobin to carry an adequate amount

of oxygen without putting an undue workload an animal hearts (Dellmann and Brown 1981).

There are several tools in cell molecular; one of these tools is Image Processing and Analysis in Java or ImageJ which is a public domain, developed at the National Institutes of Health (Collins 2007). ImageJ is a Java-based image processing program which can be used to analyze area, and pixel value statistics and intensity of user-defined pictures, as in a term of pixels per unit area. ImageJ indicates higher pixels per unit area for darker areas. When RBCs accumulate in a certain area, the color density of the area is higher than others.

3. Biological samples

RBCs of mammals are in round shape without nucleuses while RBCs of avian are in oval shape with nucleuses. Sheep RBCs are in a range of 2.5 – 3.9 μm (Dellmann and Brown 1981). White blood cells, red blood cells and platelets as the formed elements perform different functions. Inside the red blood cells, there are several substances such as hemoglobin, enzymes and ions. In the human red blood cell membrane or RBC membrane, three different types of ion channels had been described (Kaestner and Bernhardt 2002) as the well-known Voltage-gated ion chanels (Grygorczyk and Schwarz 1983, Cunningham 2002), a non-selective cation channel (NSC) (Christophersen and Bennekou 1991, Kaestner *et al.* 1999, Kaestner *et al.* 2000), and an anion channel. The anion channel is a small conductance anion channel in the RBC membrane based on different measured conductances in chloride and nitrate media (Schwarz *et al.* 1989, Passow 1988).

In red-blood-cell abnormal patients, high cations permeate in the red blood cells of patients with sickle cell disease (SCD) which occupies a central role in pathogenesis. SCD is a group of disorders that affects red blood cells. One important sickle RBC property is remarkably heterogeneous that limits conventional flux techniques, the techniques necessarily average out the behaviour of millions of the cells. Whole-cell patch configurations were investigated to characterize the permeability of single RBCs from patients with SCD in more details (Ma *et al.* 2012). A non-specific cation conductance was reversibly induced upon deoxygenation and was permeable to both univalent (Na^+, K^+, Rb^+) and also divalent (Ca^{2+}, Mg^{2+}) cations (Ma *et al.* 2012). From the diagnoses, the ion transportations can relate to sicknesses in human, as well as other living things. So, there are ions on cell membrane of RBCs.

4. Results and Discussions

The LOCs have been fabricated according to the literature (Pramuanjaroenkij *et al.* 2011). A mask for electrode fabrications was designed according to Cetin design (Cetin 2009) by using L-Edit software. In fabrication processes, a 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. A 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. The PDMS mixture was 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 holes in order to create chambers. Because electroplating technique can vary electrode surface conditions (Huang and Pethig 1991), the chromium electrode array was patterned on glass slides by only DC sputtering technique through microshadow masking. The chromium were sputtering under argon plasma with sputtering pressure, sputtering current, and time of $3x10^{-3}$ mbar, 0.2 A, and 2 minutes, respectively. The sputtering was conducted at room temperature.

4.1 Solving LOC leaking with a new acrylic-sandwich set

Only thin electrodes from the sputtering technique were used in the LOCs. In the manual assembly, the former (simple) acrylic plates (Fig. 1) were replaced by a new-acrylic-plate set as shown in Fig. 2. The LOCs from the acrylic-plate set with the second method of manual assemblies were workable; biological samples could flow through an electrical field without any electrode flooding problems.

Fig. 1 The first acrylic plates used in the previous work (Pramuanjaroenkij *et al.* 2011).

Fig. 2 A new-acrylic-plate set; H shape, used in the current work.

4.2 Buffer-sample ratio effects on microchannel LOC flows

EDTA or Ethylene Diamine Tetra-Acetic acid is anticoagulant commonly used in animal science, so we chose this solution as the buffer solution. We mixed the buffer solution with samples; sheep blood, in 3 different ratios as 1:1, 2:1 and 3:1 by volume. The mixtures were dropped onto the LOC, 50 micrometer width under room temperature condition (26°C), we found that the 1:1 mixture showed the clearest picture, red blood cells could be observed better than the others. We noticed that higher ratios; 2:1 and 3:1, the sample flowed faster and provided unclear

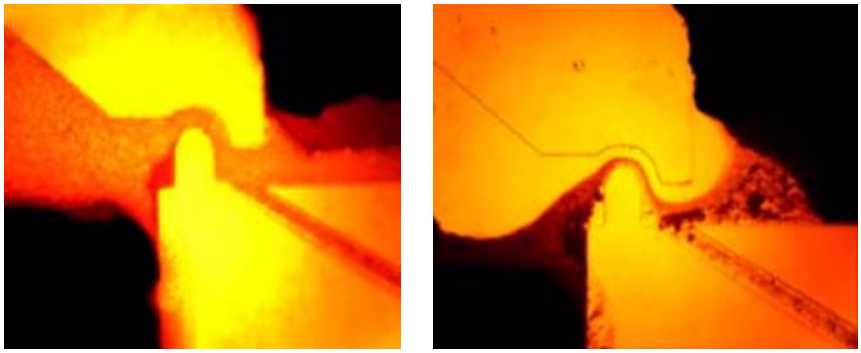
pictures.

4.3 Hematocrit results

Standard hematocrit values of sheep blood are in between 26 – 45% (Jain 1993). We examined our samples obtained from total of 6 sheep by using the traditional hematology test and found two ranges of hematocrit values from the samples; 17.8 – 22% or lower standard hematocrit and 26 – 28% or standard hematocrit. From the hematocrit ranges, we distinguished the samples to be 1) and 2) groups.

4.4 LOC-Blood-flow image analyses with the "imageJ" program

Since sheep red blood cells are very small, an image analysis was occupied to help distinguishing the flow on LOC, the sample picture as shown in Fig. 3. Results from both hematocrit groups are shown in Table 1. Using ImageJ with the images obtained from anode and cathode areas, we found that average-difference-intensities per unit area between both electrodes were 39.485 and 14.641 pixels for the standard and lower standard hematocrit groups, respectively. We noticed that the lower standard hematocrit group provided higher pixels (darker pictures) than those of the standard hematocrit group but the differences between the intensities of both electrodes were high (39.485 in average) in the standard group and low (14.641 in average) in the lower standard group. From the results, when the lower standard hematocrit samples flowed on the LOC, the RBCs were induced to both electrodes more than the standard group.



(a) (b)

Fig. 3 Sample flow patterns on the Lab-on-a-Chip; (a) standard hematocrit and (b) lower standard hematocrit.

5. Conclusions

We found that the suitable ratio between EDTA and blood was 1:1 by volume because the best image result was obtained. The intensities of sample pictures were calculated from subtractions between intensities at anode and those at cathode, the mean different color intensities per unit area were 39.485 and 14.641 pixels per unit area for the standard hematocrit and lower standard hematocrit groups, respectively. So, relationships between hematocrit quantities and the sample-

color intensities per unit area at the electrodes showed that the manual LOC coupled with "ImageJ" can be used to investigate sheep blood; especially, this coupled technique consumed less time than the traditional hematology.

Table 1 Hematocrit and mean different intensities per unit area of the samples.

Sample No.	hematocrit	Different intensities per unit area (%)	Mean different intensities per unit area (%)
N1	Standard	34.978	39.485
N2	Standard	39.163	
N3	Standard	44.315	
N4	Lower standard	10.678	14.641
N5	Lower standard	11.132	
N6	Lower standard	22.114	

Acknowledgments

The research described in this paper was financially supported by the Faculty of Natural Resources and Agro-Industry, the Faculty of Science and Engineering and the Research and Development Institute of Chalermphrakiat Sakon Nakhon Province Campus, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus.

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