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Cytometry Microchip Using Polymer-based Saltbridge Electrodes

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Abstract—**We developed a cytometry glass chip using polymer-based saltbridge electrodes. Saltbridge electrodes were placed at lateral sides of channel to increase the sensitivity and robustness of detection. The dimension of saltbridge electrodes which are exposed to channel was 50μm by 20μm(width, height). The saltbridge electrode was formed at a specific position in a predefined shape by photo polymerization technique. UV light sensitive monomer DMAC(dially dimethyl ammonium chloride) was used for polymerization. Polymer-based saltbridge electrode enabled the DC impedance analysis of the channel for cytometry. Moreover, the lateral positioning of saltbridge electrode is easily accomplished by photo polymerization technique. We validated the detection sensitivity of developed chip using 10μm fluorescent bead.**

Keywords—**Cytometry, μTAS, saltbridge, cell counter**

I. Introduction

Cell counting is important from the clinical point of view. Most of flow cytometer used in hospital utilize Coulter counting method or optical method. Coulter counter detects cells in flow by impedance change between electrodes. Optical cytometer detects cells in flow by laser scattering and fluorescent signal. High speed optical cytometer achieves the counting performance of 40,000 cells/sec. Although the speed of cytometer per se is fast enough, there is time delay between blood sampling and examination due to the procedure of sample collection, transportation and loading. This time delay is usually from several hours to 2 days. But, there are several situations that this time delay is critical. One of this situation is stem cell separation. Stem cells are separated from peripheral blood by in-vitro circulation. To obtain stem cells as much as possible, in-vitro circulation should be performed when the amount of mobilized stem cell in peripheral blood is maximally increased after injection of cytokine. To confirm that moment, one should be able to count stem cells in peripheral blood on the spot. Another situation is performing WBC(white blood cell) level control in blood of juvenile leukemia patient. In controlling the level of WBC, it is important to inject cytokine at proper time. The moment of injecting cytokine is decided by counting the neutrophil in peripheral blood. Therefore one could determine proper time by counting neutrophil in peripheral blood on the spot.

In these cases, POCT(point of care test) cytometry would be helpful. So, the implementation of small-size cytometry chip is important.

In previous research, most of cytometry techniques were based on AC impedance analysis between co-planar metal electrodes[1]. To increase sensitivity of detection and robustness in diverse position of cells in channel, electrodes should be placed in parallel at each lateral side of channel. But, vertical electroplating of channel is difficult. Another work suggested lateral thick metal electrodes by bulk electroplating of gold[2]. In these cases of metal electrode, impedance analysis of interrogated region between electrodes should be performed at high frequency AC current, because DC current causes oxidation/reduction reaction on the surface of metal electrodes. And air bubbles caused by the reactions on the surface of metal electrode obstruct the flow.

In this study, a cytometry technique was developed using polymer-based saltbridge electrodes. It has two advantages over metal electrode. First, it is easy to place saltbridge electrodes at lateral sides of channel. This increases the sensitivity and robustness of detection. Second, DC current can be used for the impedance analysis of channel because there is no oxidation/reduction reaction on the surface of saltbridge electrodes. This simplifies the analysis system and enables the implementation of small-size cytometry device.

II. Methodology

A. Glass chip fabrication

Fig. 1 shows the fabrication process of micro channel glass chip. Corning 2947 slide glass(75mm by 25 mm, 1mm thick) is used as substrate. Slide glasses are cleaned with piranha solution, acetone, methanol and DI water. Cleaned glass is dehydrated on hot plate at 150°C for 10 min. HMDS is spin coated on glass at 4,000rpm for 30 sec. Photo resist AZ5214-E is spin coated on glass at 4,000rmp for 30 sec. Soft baking of PR is performed on hot plate at 100°C for 1 min. Exposure of UV light(365nm) with intensity of 20mW/cm^2 is performed for 6.5 sec. PR is developed with AZ300MIF for 45 sec. Hard baking of PR is performed on hot plate at 100°C for 15 min. Glass etching is performed with 6:1 buffered oxide etch solution for 30 min. Glass is cleaned with acetone, methanol and DI water. Another slide glass which is drilled at reservoir is bonded to etched slide glass by thermal bonding(590°C, 6 hours). The dimension of fabricated channel is 50μm by 20μm(width, height). Fabricated glass chip is shown in Fig. 2. Saltbridge electrodes are formed at tear-drop like region in enlarged picture.

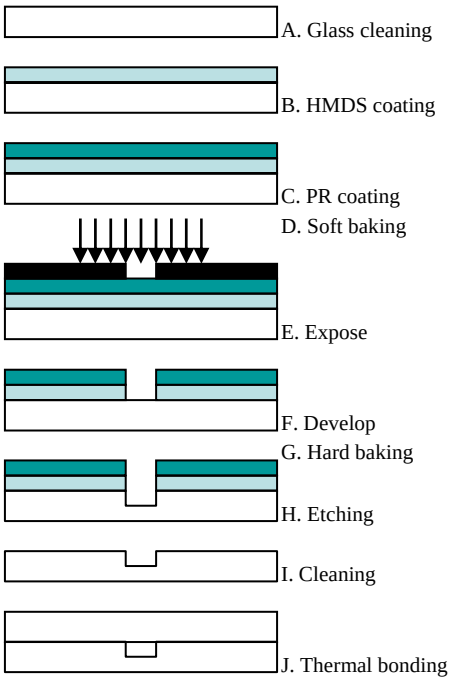

Fig. 1. Glass chip fabrication. A. Slide glass is cleaned with piranha solution, acetone, methanol and DI water. Cleaned glass is dehydrated on hot plate at 150°C, 10 min. B. HMDS is spin coated at 4,000rpm, 30 sec. C. AZ5214-E is spin coated at 4,000rmp, 30 sec. D. Soft baking on hot plate at 100°C, 1 min. E. Exposure of UV light(365nm) with intensity of 20mW/cm^2 for 6.5 sec. F. Develop PR with AZ300MIF for 45 sec. G. Hard baking on hot plate at 100°C, 15 min. H. Etching with 6:1 buffered oxide etch solution for 30 min. I. Cleaning with acetone, methanol and DI water. J. Another slide glass which is drilled at reservoir is bonded to etched slide glass by thermal bonding(590°C, 6 hours). The dimension of channel is 50μm by 20μm(width, height).

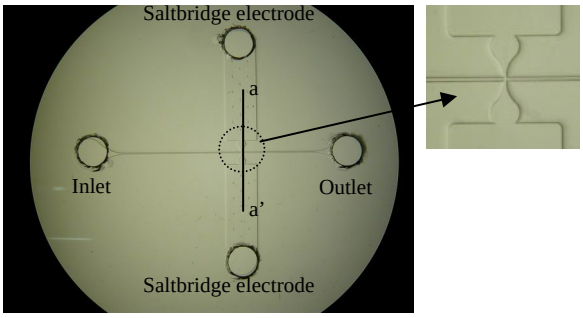

Fig. 2. Fabricated glass chip. Sample solution flows from left to right. There are two saltbridge electrodes at lateral sides of channel. This parallel position of electrodes increases the sensitivity and robustness of detection.

B. Saltbridge fabrication

Fig. 3. shows the fabrication process of saltbridge electrodes. Photo polymerization technique was used to form saltbridge electrodes at specific position with predefined shape. Positively charged and UV light sensitive monomer DMAC is diluted in water to 65 wt%. DMPAP(2.2-Dimethoxy-2-phenyl-acetophenone) is added for photo initiation of polymerization process. The density of DMPAP is 1 mol% with respect to DMAC. KCl is added as salt to 1M. The glass chip is filled with the monomer solution and aligned under photo mask. Exposure of UV light(365nm) with intensity of 20mW/cm^2 is performed for 3 min. Then DMAC monomer solution is polymerized to PDMAC(poly-DMAC). Channel is cleaned with 1M KCl solution.

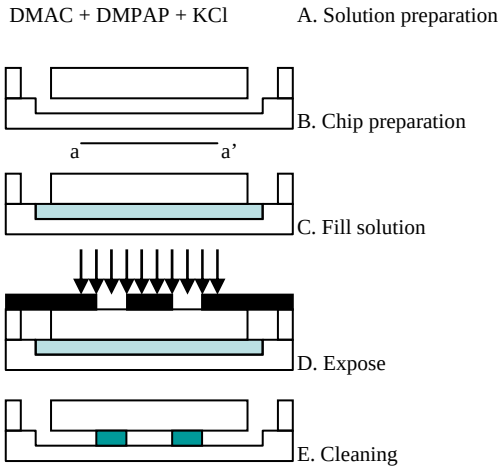

Fig. 3. Saltbridge fabrication. A. Solution preparation. Positively charged and UV light sensitive monomer DMAC is diluted in water to 65 wt%. DMPAP(2.2-Dimethoxy-2-phenyl-acetophenone) is added for photo initiation of polymerization process(1 mol%). KCl is added as salt(1M). B. Cross section of glass chip with cutting line aa' in Fig.2. C. Fill the channel with monomer solution. D. Exposure of UV light(365nm) with intensity of 20mW/cm^2 for 3 min. E. Cleaning with 1M KCl solution.

C. Cytometry by impedance analysis

Fig. 4. shows cytometry method by DC impedance analysis using saltbridge electrodes. Salt bridge electrodes are connected to the analysis system by Ag/AgCl electrodes and 1M KCl solution. DC potential source applies 0.4V between two Ag/AgCl electrodes. The current signal is amplified and stored on PC.

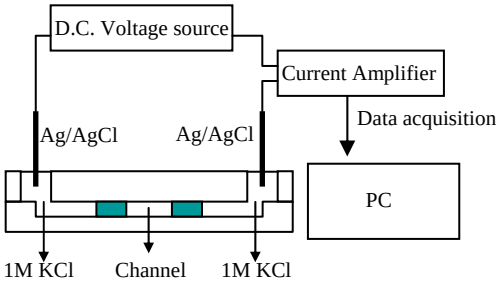

Fig. 4. DC impedance analysis of channel for cytometry using saltbridge electrodes. Ag/AgCl electrodes and 1M KCl solution are used for the connection of saltbridge electrodes to analysis system.

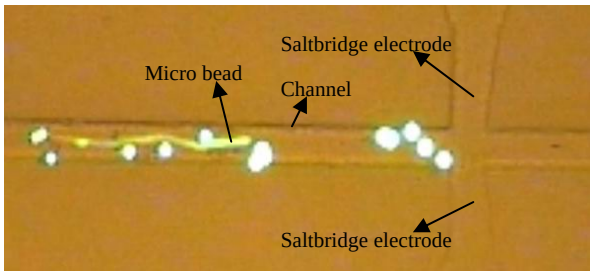

Fig. 5. Flow of fluorescent bead through channel. PDMAC saltbridge electrode is almost transparent when it is wet.

D. Performance Evaluation

10μm fluorescent beads are used to evaluate the performance of developed cytometry chip. 10μm fluorescent beads are diluted in 10mM borate buffer to 0.1%. Flow is generated by pneumatic pump. Fig. 5 shows the flow of fluorescent bead through channel.

III. RESULTS

Fig. 6 shows current signal obtained by the experiment above. DC current fluctuates when fluorescent beads pass the interrogated region between two saltbridge electrodes. The amplitude of fluctuation is almost uniform. The width of fluctuation signal varies because the flow generated by pneumatic pump was not stable.

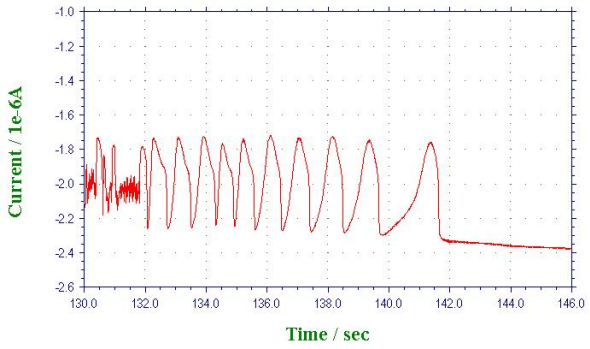

Fig. 6. Current signal when 10μm fluorescent beads pass the interrogated region between saltbridge electrodes. The amplitude of fluctuation is almost uniform.

IV. CONCLUSION

A cytometry technique was proposed based on saltbridge electrodes. The unique feature of DC impedance analysis simplifies the whole system and will contribute to the implementation of POCT cytometry. Performance evaluation using fluorescent bead confirms the possibility of the system as an effective tool for cytometry..

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