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Enhanced Fluorescence by Controlled Surface Roughness of Plastic Biochip

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Fluoroimmunoassay is one of the protein detection methods in which the most critical parameter is fluorescence intensity as it determines the sensitivity of the analysis. In this study, cyclic olefin copolymer (COC) based plastic biochips of various thicknesses were fabricated from 304 SS (Stainless Steel) molds using imprinting technique. The effect of surface roughness of COC biochip on the enhancement of fluorescence intensity was investigated. The fluorescence intensity reached the maximum value at optimum value of surface roughness without significantly affecting the auto-fluorescence value. The process proposed in this technique is simple, low cost yet highly sensitive for protein detection.

1. Introduction

Biochip such as protein, DNA, neuron, and cell chip made of solid substrate of few micrometer to millimeter size has been used for medical diagnosis, food inspection and biomedical research.

Biochip has certain advantages over other pre-existing analytical methods such as increased portability, accuracy, and rapid analysis. Thus, biochips have been gaining interest in various research areas such as bio analysis, new medicine discovery and diagnosis.[1,2)]

In the recent years, plastic replaces glass as a substrate material in the fabrication of biochip as it has merits when compared to glass substrate such as cheap, light, flexible and easy fabrication though it also has demerits such as low fluidity and difficulty in immobilization of bio contents due to its hydrophobic nature. However, these issues could be overcome by surface modification of plastic substrate from hydrophobic to hydrophilic nature.[3–6)] Thus in the present work, plastic biochip with hydrophilic surface was fabricated from 304 SS mold using imprinting technique.[7)]

Generally, analytical biochip methods can be classified into electro-chemical method,[8)] Enzyme-linked immunosorbent assay (ELISA)[9)] and fluoroimmunoassay.[10)] Among these methods, fluoroimmunoassay is widely used as a bio analytical technique because it is simple, cheap and high throughput screening. Fluoroimmunoassay method analyzes fluorescence intensity results from the immune reaction of antibody with antigen which is conjugated with fluorescence molecule.

Fluorescence has long been used in biotechnology due to its advantages such as high sensitivity, easy handling, lower cost and large dynamic range detection. There have been many studies to enhance the fluorescence signals such as resonant coupling between excitations and surface plasmon of metal,[11–13)] and nano particles for high sensitivity of antigen–antibody by increased surface roughness.[14–16)] Those methods require complicated structure, delicate manipulation and high cost. Long detection time and low reliability also made them difficult to adapt in practical applications. Thus the purpose of this present work is to propose a simple strategy to enhance the fluorescence signals by controlling the surface roughness of the plastic biochip. The effect of surface roughness on the fluorescence intensity was investigated via fluoroimmunoassay.

2. Experiments

Prior to fabrication of 304 SS mold, 304 SS wafer of 6-in. diameter was first polished and then cleaned with high purity isopropyl alcohol (IPA) 99.8% for 3 min using ultrasonic cleaner to remove organic residues. Figure 1(a) shows the schematic illustration of electrochemical fabrication (ECF) mold fabrication.[17)] Photolithography process was used to fabricate pattern on the substrate. In the first step, positive photoresist (Clariant AZ1512) was spin-coated on the substrate surface at 3000 rpm for 30 s and was baked on the hot plate at 100 °C for 1 min. The coating thickness of photoresist material was 1 μm. UV aligner (EVG620) was used to make patterns on 304 SS wafer. The photoresist was exposed to broadband wavelength ($365 < \lambda < 436$ nm) of aligner for 6 s. After exposure, photoresist on the 304 SS wafer was developed using commercial developer (Clariant AZ300MIF).

ECF method was used to fabricate micro structures on 304 SS. In this method, patterned 304 SS wafer acts as an anode for etching whereas copper acts as a cathode. Both these electrodes dipped in the electrolyte solution consisted of H_2SO_4, H_3PO_4, deionized water (DI-W) and additives. The electrolytic solution is continuously stirred using the stirrer. The direct current (DC) of constant density was applied to the electrode terminals by a rectifier. Patterned 304 SS wafer was allowed to etch via oxidation reaction. The surface roughness was controlled by dipping the mold in 97% $FeCl_3$ solution[18)] (Junsei Ferric chloride) as a function of time as shown in Fig. 1(b). The roughness of surfaces was analyzed by stylus profilometer (Veeco Dektak-6M).

Cyclic olefin copolymer (COC) was chosen as a substrate material in the present work due to its high transparency, high chemical resistivity, high stiff material and high glass transition temperature (T_g). 30 nm thick fluorocarbon (FC) anti-stiction layer was coated on surface of 304 SS by FC-chemical vapor deposition (CVD; Sorona SRN-504) by using C_4F_8. Imprinting was performed to duplicate the COC biochips by using thermal imprint machine (KIMM ANT-4) from 304 SS mold at 150 °C and 150 kg for 5 min. A 30-nm-thick fluorocarbon (FC) anti-stiction layer was coated on its surface by FC-CVD (Sorona SRN-504) by using C_4F_8.

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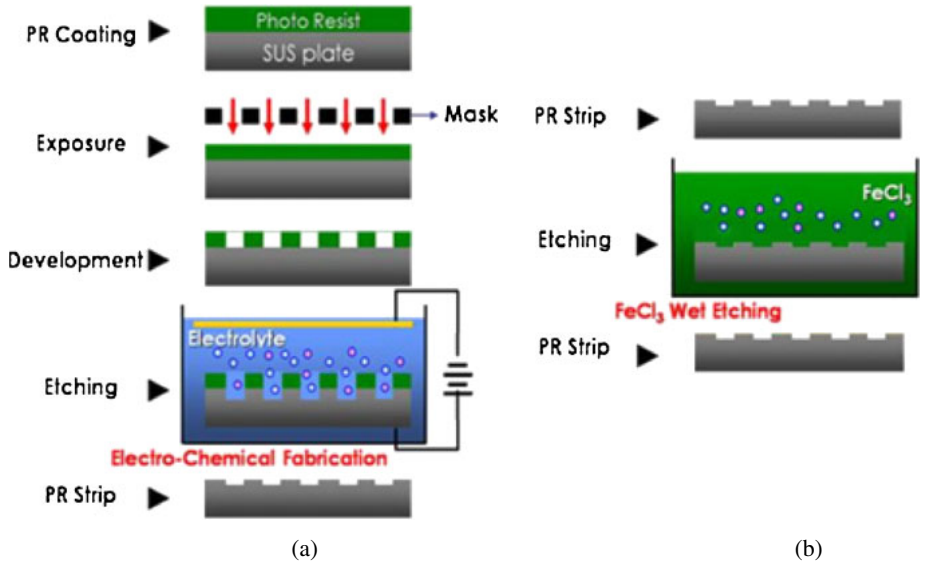

Fig. 1. (Color online) Schematic illustration of fabrication of 304 SS mold. (a) ECF process and (b) $FeCl_3$ process.

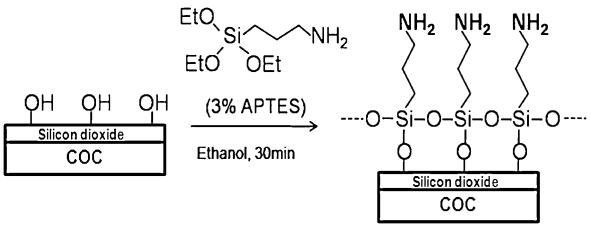

Fig. 2. A schematic illustration of amine surface modification on COC.

The surface of COC biochip was modified to hydrophilic by depositing silicone dioxide film 1000 Å by plasma enhanced CVD (PE-CVD; Oxford Lab80Plus) using 1000 mTorr of SiH_4 170 sccm and N_2O 710 sccm at 70 °C. Then, oxide deposited COC was soaked in the 3% (3-aminopropyl)triethoxysilane (APTES; 99%, Sigma) solution (dissolved in 97% ethanol) for 30 min for self-assembled monolayer layer (SAM) formation followed by rinsing in ethanol and DI-W for 3 min with ultrasonication to remove physically adsorbed molecules on the surface. The hydrophilicity of the surface was measured using contact angle analyzer (SEO Pheonix300). Figure 2 shows a schematic illustration of surface modification of COC surface.

The fluorescence intensity of the fabricated biochip with amine group on the surfaces was measured via fluoroimmunoassay. In fluoroimmunoassay, fluorescence intensity was measured through specific antibody, antigen and CY-5 dye molecule conjugated with antibody sandwich structure. Figure 3 shows a schematic illustration of H1N1 fluoroimmunoassay process. In this study, anti influenza A and H1N1 used as an antibody and antigen, respectively. In the first step, 1.5 µl droplet of anti influenza solution was allowed to react with amide group of COC surface for 30 min in darkroom condition and then washed with phosphate buffered saline (PBS) buffer flow and DI-water before drying with N_2 blow. During this step anti-influenza A was adhered with NH_2 group of COC surface. In the second step, antigen H1N1 was immobilized to anti-influenza A with 1.5 µl droplet for 30 min in dark room. Then finally, CY-5 conjugated anti-influenza was captured by H1N1 antigen immobilized surface. The emission fluorescence signal from this sandwich structure was acquired as a function of surface roughness of substrate. In addition to the fluorescence intensity, auto-fluorescence emitted from the polymer was also measured as a function of surface roughness. Since auto-fluorescence level of the COC increases the background signal and interferes with the detection of specific fluorescent signal.

3. Results and Discussion

The 304 SS mold was fabricated by ECF process. To change the roughness of 304 SS mold, the mold was etched in $FeCl_3$ etchant as a function of time. Roughness of etched 304 SS mold is increased with increasing etch time as shown in Fig. 4(a). The surface roughness of 304 SS mold untreated with $FeCl_3$ etching (pristine) is 0.05 µm and increased to 1.4 µm with respect to etching time. Then the surface roughness of 304 SS mold is transferred to COC biochip through imprinting technique and the results are shown in the Fig. 4(b). In this case, the surface roughness is increased from 0.2 to 2.25 µm.

Contact angle measurement of amine modified COC substrate with DI-Water was carried out to confirm the surface condition. Figures 5(a) and 5(b) show the effect of silicon oxide thickness on contact angle and fluorescence intensity. There was no significant change in contact angle as a function of silicone oxide thickness due to its film nature determined by gas precursor as shown in Fig. 5(a). In Fig. 5(b), there was a 2-fold increase in the fluorescence intensity as the oxide thickness increased from 2500 to 5000 Å.

Figure 6 shows measured fluorescence intensity as a function of COC surface roughness. The increase in surface roughness amplified the fluorescence signal. This is because the increase in surface roughness increases the surface area of the substrate and thereby results in the increase of the number of fluorescence molecules adsorbed on the surface.

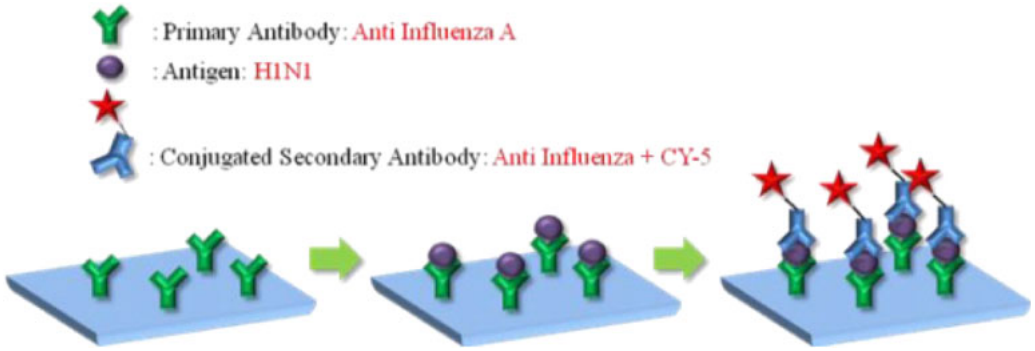

Fig. 3. (Color online) A schematic illustration of H1N1 fluoroimmunoassay process.

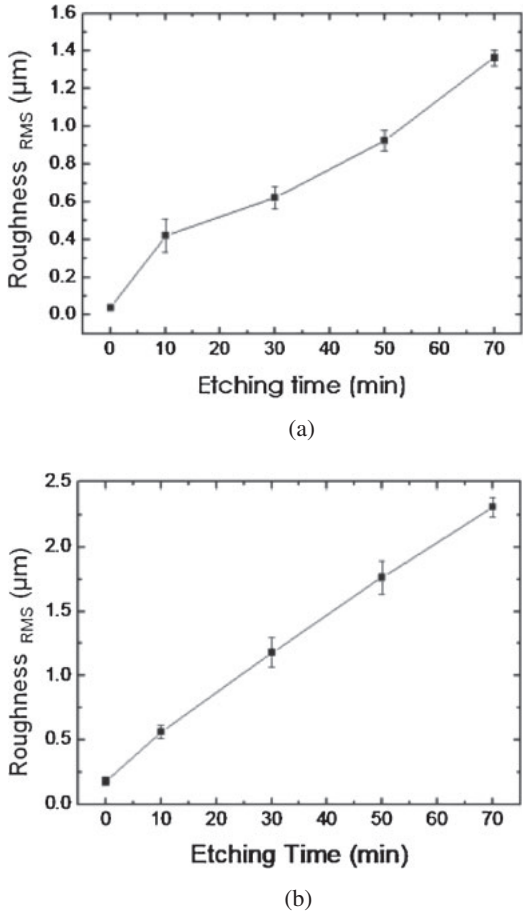

Fig. 4. Surface roughness (a) SS 304 as a function of $FeCl_3$ etching time and (b) roughness of imprinted COC surface with 1000-Å-thick silicon dioxide.

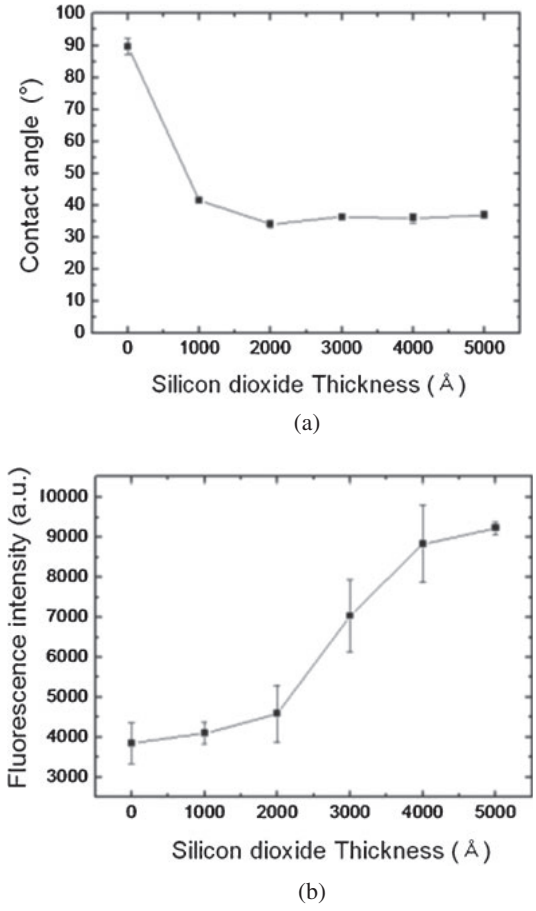

Fig. 5. The effect of thickness of silicon oxide on (a) contact angle and (b) fluorescence intensity.

It can eventually result in the amplification of fluorescence signal. However, there should not be any change in the auto-fluorescence signal of the substrate when the surface roughness increases as this interferes with fluorescence emission signal and reduce the sensitivity of the diagnosis. To confirm this, the auto-fluorescence of the COC biochip was also measured using fluorescence scanner. Figure 6(a) shows the effect of surface roughness on the auto-fluorescence. It clearly shows that there is no significant change in the auto-fluorescence as it is only varied from 115 to 140 a.u. when the surface roughness increases from 0.2 to 0.3 μm.

Figure 6(b) shows the variation of fluorescence intensity measured using fluoroimmunoassay as a function of surface roughness of COC biochip. The plot clearly shows that fluorescence intensity enhanced significantly from 500 to 2300 a.u. This change is relatively higher when compared to the change of auto-fluorescence. Thus it could be concluded that intensity of auto-fluorescence would not interfere with the fluorescence intensity on rough surface and thereby the sensitivity of the diagnosis. It is also observed from Fig. 6(b) the maximum intensity is achieved even with the surface roughness of 1.2 μm and further increase in surface roughness does not affect the fluorescence intensity value significantly.

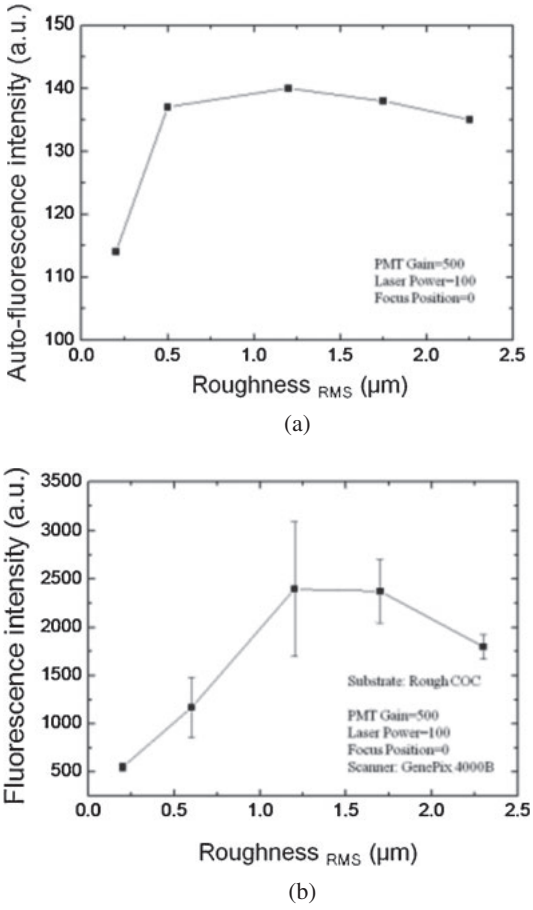

Fig. 6. The effect of surface roughness on (a) auto-fluorescence and (b) fluoroimmunoassay with H1N1 influenza.

4. Conclusions

In this work, the plastic (COC) biochip was duplicated from 304 SS mold using imprinting technique. The fabricated 304 SS mold was etched by $FeCl_3$ etching and the surface roughness of the 304 SS mold and thereby the COC biochip was controlled by etching time. The effect of surface roughness on the fluorescence signal was investigated. Fluoroimmunoassay shows that the fluorescence signal intensity is amplified with the increase in surface roughness to the optimum value without significantly affecting the auto-fluorescence intensity. The signal amplification is mainly due to the increase in surface area which eventually increases the number of fluorescence molecules adsorbed on the surface. Hence this simple and low cost strategy of controlling surface roughness of COC biochip to the optimum value could be used to amplify the fluorescence intensity signal as it enhances the sensitivity of the protein detection analysis.

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