Fabrication of Plastic Optical Label-free Biosensor and Immunodetection of Cardiac Marker in Human Serum

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ABSTRACT

We have studied for lab-on-a-chip (LOC) based bio-devices and fabricated plastic optical label-free biosensor device. Optical resonant reflection biosensor (ORRB) is a kind of label-free sensor, containing nanoscale resonate grating patterns[1]and whole blood filter. This plastic ORRB sensor is fabricated with cyclic olefin copolymer (COC) via conventional injection molding method.

Injected ORRB chips employ a top plate and a bottom plate. The top plate incorporates a microfluidic channel for humam serum and the bottom plate has a sensing region, which consists of a nano-pattern, and a fluidic velocity control region referred to as a time-gate. The sensing region is formed by deposition of a high refractive index layer, Si_3N_4, by a plasma enhanced chemical vapor deposition (PECVD) technique. After the ORRB chip is treated by oxygen plasma in order to render it hydrophilic, the top and bottom plates are bonded by ultra sonic equipment without application of any glue.

In this paper, we mention the properties of injection molding plastic ORRB sensor chip with pretreatment process, which are Si_3N_4, deposition conditions, immobilized process condition on plastic ORRB sensor and also experimental immunodetection results of cardiac markers in human serum. We characterized peak wavelength shifting cause of difference of optical density and refractive index from antigen-antibody interactions results.

Keywords: Biosensor, Injection molding, Plastic biochip, Nano-pattern replica

1 INTRODUCTION

Generally, bioassay technologies can be divided into labeling methods and label-free methods. Labeling methods compounds such as fluorescent, radioactive, or colorimetric target particles are attached to a target protein such as an antibody but label-free methods do not used with any kinds of targets or particles. Even though labeling methods offer high sensitivity, they also suffer from costliness, and slow procedures, and also need to huge sensing equipment. Therefore, label-free techniques have interested in these days by several research groups, which have studied label-free biosensors such as surface plasmon resonance (SPR) [2], and photonic crystal based biosensors[3]. Currently, label-free biosensors have large chip to chip variations, and a time-consuming process due to laboratory level production. In this paper, we focused on minimizing quality variation of label-free biosensors, especially optical biosensors, by means of injection molding technique, which is one of the most population method for commercial lab–on a chip (LOC) or point of care (POC) kits.

Injection molding methods to fabricate plastic biochips are the most effective tool to reduce production cost [4-8] and to improve the stable performance of chips. In biotechnology and medical fields microstructure fabrication has become common, such as a micro fluidic channel, a micro-mixer, and micro-reactors. Biochips with surface microstructure are expected to provide several advantages by providing enhanced efficiency, and sensitivity as well as time-savings through the use of large volumes in the analysis[9].

In our case of the injection molding technique, a nanosize pattern should be formed on a plastic biochip surface using a metal mold. We successfully fabricate a new label-free optical biosensor made entirely of a plastic material using a conventional injection molding method. A highly sensitive and economical biosensor having a compact design is realized via this approach. We also discuss the properties of the label-free optical biosensor chip and provide detailed processing results and experimental immunodetection results of cardiac markers in human serum.

2 EXPERIMENT

The new label-free optical biosensor developed in this study, referred to as an optical resonance reflection biosensor (ORRB), utilizes a sub-micron size grating structure. The grating reflects only a very narrow resonant peak when the white light is illuminated at the normal direction of sensor surface. To design a very sharp resonance peak from the ORRB chip, we simulate a two-dimensional nanosize grating structure using a diffraction grating analysis tool (GSOLVE) employing the rigorous coupled wave theory. Further details of the simulation technique and the relevant theory can be found in a previous report by the authors[10]. GSOLVE provides a quick and simple result for the initial 2D nanosize grating. Figure 1is a schematic the grating structure of optical resonance reflection biosensor (ORRB) structure[11].

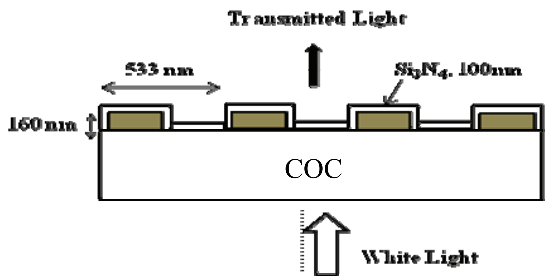

Figure 1. The schematic design of optical resonance reflection biosensor (ORRB) under the resonant conditions.

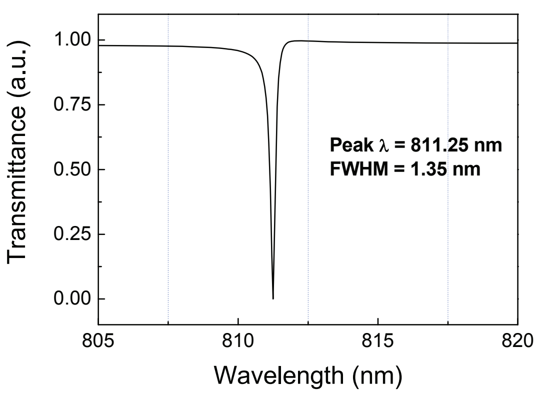

Figure 2. The resonant peak of the designed ORRB exposed to PBS, as simulated by GSOLVE.

Figure 2 presents the results of the simulation for the ORRB chip. Calculated a resonant peak wavelength of about 811 nm from the ORRB at Si_3N_4 = 100 nm, the refraction index of COC, phosphate buffered saline (PBS), a UV curable polymer, and Si_3N_4 is a 1.475, 1.335, 1.5, and 2.0, respectively. From figure 1, it is seen that the grating period is 533 nm (land: groove = 6:4) and the height of land 160 nm (± 10nm).

The Ni stamp, which is a replication mold for injection with plastic, is fabricated by an electroplating method using ICP etched nano-pattern on the Si wafer, which is made by nanoimprint machine such as an IMPRIO 55(MII). And then etching process is applied in order to remove the residual layer, that is, excluding the region of the nano-pattern, of UV polymer on the Si wafer with induction coupled plasma (ICP) machine. Various gases such as SF_6, CHF_3, Ar, and O_2 are employed for ICP with different RF power and working pressure.

The electroplating method generally entails the deposition of a metal seed layer, (in the present case, a 100 nm Cr layer) on the imprinted nano-patteren by e-beam evaporation. Ni electroplating in a Ni $(NH_2SO_3)_2$ $4H_2O$ + $NiCl_2$ $6H_2O$ + H_3BO_3 solution is performed under an electric field. A high quality and uniformed Ni stamp, including a nano-pattern, is made by precisely controlling the stress conditions such that they are minimized during the electroplating. The thickness of the Ni stamp is approximately 500 um. The Ni stamp mount in the injection mold is specially designed for the ORRB chip but can be adapted to commercial injection equipment, such as a Sumitomo SD-35T, which is optimized for nano-pattern translation with plastic material. Table 1 shows an injection molding process conditions.

Table1. The conditions of injection equipment, Sumitomo SD-35T, for ORRB chip fabrication.

Injection Pressure (Kg/cm^2)	**Injection Speed (mm/sec)**	**Mold Temp. (℃)**	**Cooling Time (Sec.)**
272	198	100	11

The plastic ORRB chip is made of cyclic olefin copolymer (COC) for good bio-compatibility and high optical transmittance. Injected ORRB chips employ a top plate and a bottom plate. The top plate incorporates a microfluidic channel for the reagent solution and the bottom plate has a sensing region, which consists of a nano-pattern, and a fluidic velocity control region referred to as a time-gate. The sensing region is formed by deposition of a high refractive index layer, Si_3N_4, by a plasma enhanced chemical vapor deposition (PECVD) technique. After the ORRB chip is treated by oxygen plasma in order to render it hydrophilic, the top and bottom plates are bonded by ultra sonic bonding equipment without application of any glue.

3 RESULTS AND DISCUSSION

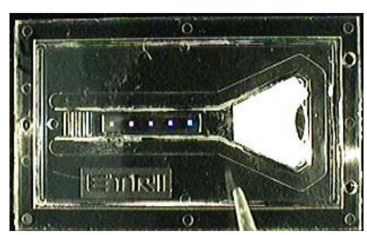

Figure 3. Picture of a nano-pattern incorporated ORRB chip by the injection molding method.

Figure 3 shows a picture of the injection molded plastic ORRB chip. A five point bright nano-grating pattern by optical diffraction can be clearly observed. From the cross sectional scanning electron microscope (SEM) image of the plastic ORRB chip (it is not shown in this paper) shows the uniformly deposited Si_3N_4 layer. From the figure 3, the size of the injection molded plastic is 25(W) mm x 44.5(L) mm x 2(T) mm and the ORRB chip has three holes of 1.5mm diameter for alignment in the chip holder of the optical detection equipment. And white colored trapezoid is whole blood filter, which is made by micro fiber type and it can filter 50ul whole blood within a minute.

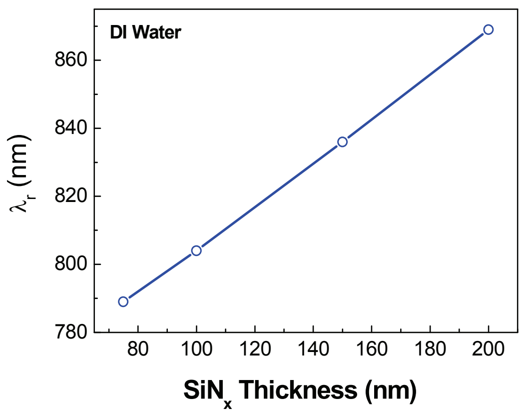

Figure 4. Resonance peak wavelength value (PWV) shift of plastic ORRB chip with different SiN_x layer thickness.

Figure 4 shows the resonance peak wavelength value (PWV) shift for the Si_3N_4 coated plastic ORRB chip. The change of the PWV shift is almost a linear curve, and this curve also indicates that the nano-grating is very regularly formed on a sensing area of the injection molded ORRB chip. The linear property of the resonant peak indicates that the influence on quality deviation of the injection molded nano-replication pattern can be neglected at the detection level. If the injection process and Ni stamp fabrication process are optimized, this performance will be improved on nanoimprint level. As a result, the quality of the nano-pattern of the plastic ORRB chip is not poor compared with that of a glass chip, where a nano-pattern is directly formed on the glass substrate via a nanoimprint lithography machine (IMPRIO 55).

After successfully fabricating the plastic ORRB chip, we measured the resonant peak shift for various solutions, having different refraction values: deionized (DI) water, phosphate buffered saline (PBS; pH 7.0), ethanol, and isopropylalcohol (IPA). The measured data is presented in figure 5. Figure 5 shows specific resonance curves for various solutions obtained with the same ORRB chip. The refraction index of deionized (DI) water, phosphate buffered saline (PBS), ethanol, and isopropylalcohol (IPA) is 1.333, 1.335, 1.360, and 1.378, respectively. From the figure 5, the resonant wavelength shift is about 0.39 nm and it obviously varies with the change of the refraction index when the refraction index is changed by 0.001.

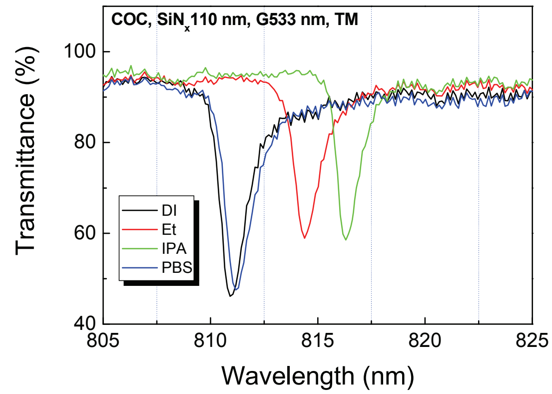

Figure 5. Resonance peak wavelength value (PWV) shift of plastic ORRB chip with different various solutions, having different refraction values: deionized (DI) water (n=1.333), phosphate buffered saline (n=1.335), ethanol (n=1.360), and isopropylalcohol (IPA) (n=1.378).

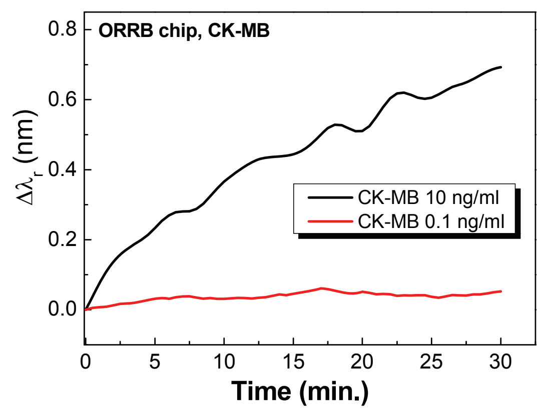

Figure 6. Time dependant peak wavelength value shift data of plastic ORRB chip with two different CK-MB concentrations, which are 10 ng/mL and 0.1 ng/mL, in human serum.

Figure 6 indicate that time dependant PWV shift of bio-molecules immobilizated plastic ORRB chip. We used the same injection mold and injection conditions for fabricating plastic ORRB chip and also simultaneously immobilize for same antibody of CK-MB. The immobilized protocol for antibody of CK-MB: 1. hydroxyl groups (-OH) forming by oxygen plasma treatment (30pa, oxygen 100ml/min, 300 seconds and 100 W) 2. Amino functional group is formed by silanization with APTES. 3. Rinsed with ethanol, and baked at 120℃ 4. Reactive aldehyde (CHO) group is formed with GA. as a bifunctional cross-linking agent, which could be coupled with anti-CK-MB. 5. The ORRB chips were then rinsed with DI water, and dried with nitrogen blow. Measurement set-up applied to figure 1 structure, therefore, we used white light source, broad band source, transmission type, which means chip position is over the light source, and used high resolution spectrometer. We drop the human serum, where two kinds of CK-MB antigen include, into the antibodies of CK-MB immobilizated plastic ORRB chip. From the figure 6, the time dependant PWV shift is about 0.7 nm in case of 10 ng/ml of antigen of CK-MB concentration. And in case of 0.1ng/ml, PWV shift is negligible. As a result of time dependant PWV shift of injection molded plastic ORRB biochip is clearly discriminate between low antigen concentration and high one. Therefore, our plastic ORRB chip can be applied to bio-sensing tool for cardiac marker, CK-MB. And this graph also proves the possibility of application of plastic based label-free optical biochip including nano-pattern and mass production in low price using a commercial injection molded machine (Sumitomo SD-35T).

4 CONCLUSION

We successfully designed and fabricated a plastic ORRB chip by a commercial injection molding technique using a sub-micron patterned Ni stamp. We also detected a refraction index variation of 0.001 by measuring the difference of resonantly transmitted wavelengths for deionized (DI) water, phosphate buffered saline (PBS), ethanol, and isopropylalcohol (IPA). From the cardiac marker, CK-MB results, our injection molded plastic ORRB chip can provide very precise bio-sensing kit. It can be clearly discriminate between 10ng/ml and 0.1 ng/ml of CK-MB antigen concentration for the measurement time, 30 minutes. This results and fabrication technique shows great promise for cardiac diseases as well as extension to other ones and applications involving biosensors or medical equipment.

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REFERENCES

[1] K.H. Kim, C. Huh, J.C. Hong, H.S. Ko, W.J. Kim, and G.Y. Sung, 2008 MRS spring meeting Abstract No.BB2.3., San Francisco.

[2] R. Karlsson, and R. Stahlberg, Analytical Biochemistry, 228, 274, 1995.

[3] I.D. Block, L.L. Chan, and B.T. Cunningham, Sensors and Actuators B, 120, 187, 2006.

[4] A. K. Angelv and J. P. Coulter, SPE ANTEC Tech., 748, 2004.

[5] C. J. Hwang, Proceeding The Japan-Korea Plastics Processing Joint Seminar, 59, 2004.

[6] Ito. Y. Kurahashi and T. Uchida, D. Omron technics 34(4), 286, 1994.

[7] T. Yasuhara, H. Imamura, K. Sawamoto, K. Kato, and H. Ohtake, Proceeding The Japan-Korea Plastics Processing Joint Seminar, 59, 2004.

[8] C. Yoshimura, Y. Kobayakashi , H. Aoki, H. Ueno, and S. Sugiyama, Trans. IEE of Japan, 121 E(5), 266, 2001.

[9] W. Michaeli, R. Gartner and D. Opfermann, Proceedings of PPS-21, SL2.1, 2005.

[10] Jongcheol Hong, Kyung-Hyun Kim, Jae-Heon Shin, Chul Huh, and Gun Yong Sung, Optics Express, 15(14), 8972, 2007.

[11] Bong Kyu Kim, Kyung-Hyun Kim, Jongcheol Hong, Wan-Joong Kim, Hyunsung Ko, Chul Huh, Sun Hee Park, and Gun Yong Sung, Applied Optics, in press, 2010.