ELECTRONIC MICROFLUIDIC BIOCHIPS WITH IMMUNE-LIKE BIOSENSORS FOR RAPID DETECTION OF C-REACTIVE PROTEIN IN HUMAN SERUM

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ABSTRACT

In this paper, novel electronic microfluidic biochips with on-chip immune-like biosensors for rapid detection of C-reactive protein in human serum have been designed, fabricated, and characterized. According to the measurements, the specificity of PIP films was up to 450%, which is the same as that of biological antibodies. The adhesion force between PIP films and antibody-coated AFM tips showed no decrease at least for 3 months. The linear range of the developed microfluidic microchip with PIP biosensors is from 2 μg/ml to 200 μg/ml for human serum samples. The response time of the developed biosensor is around 110 seconds.

KEYWORDS

Microfluidic biochip, immune-like biosensor, c-reactive protein, electrical detection.

INTRODUCTION

Biomolecular recognition layer is one of the key components of biosensors, especially for protein sensing. Most of protein biosensors use biological antibodies as biomolecular recognition layers [1], which have the disadvantages of short lifetime, short shelf-life at elevated temperature, and poor chemical resistance. In this abstract, microfluidic biochips with immune-like biosensors are developed. The immune-like biosensors consist of nanocavities on the surfaces, which have high specificity and long shelf-life, for capture of target protein in samples.

DESIGN AND FABRICATION

Figure 1 shows the design of our electronic microfluidic biochips with immune-like biosensors for rapid detection of c-reactive protein in human serum. In this study, we unify the orientation of template molecules during imprinting process by binding CRP antibody on the template, which would enhance specificity of the biosensors. The developed technique polymerized and patterned the protein-imprinted polymer (PIP) biosensor with designed photomasks by UV exposure process, as shown in Figure 2. After the templates extraction process, the developed PIP films on the chips can be used to identify and separate the target molecules, as shown in Figure 3.

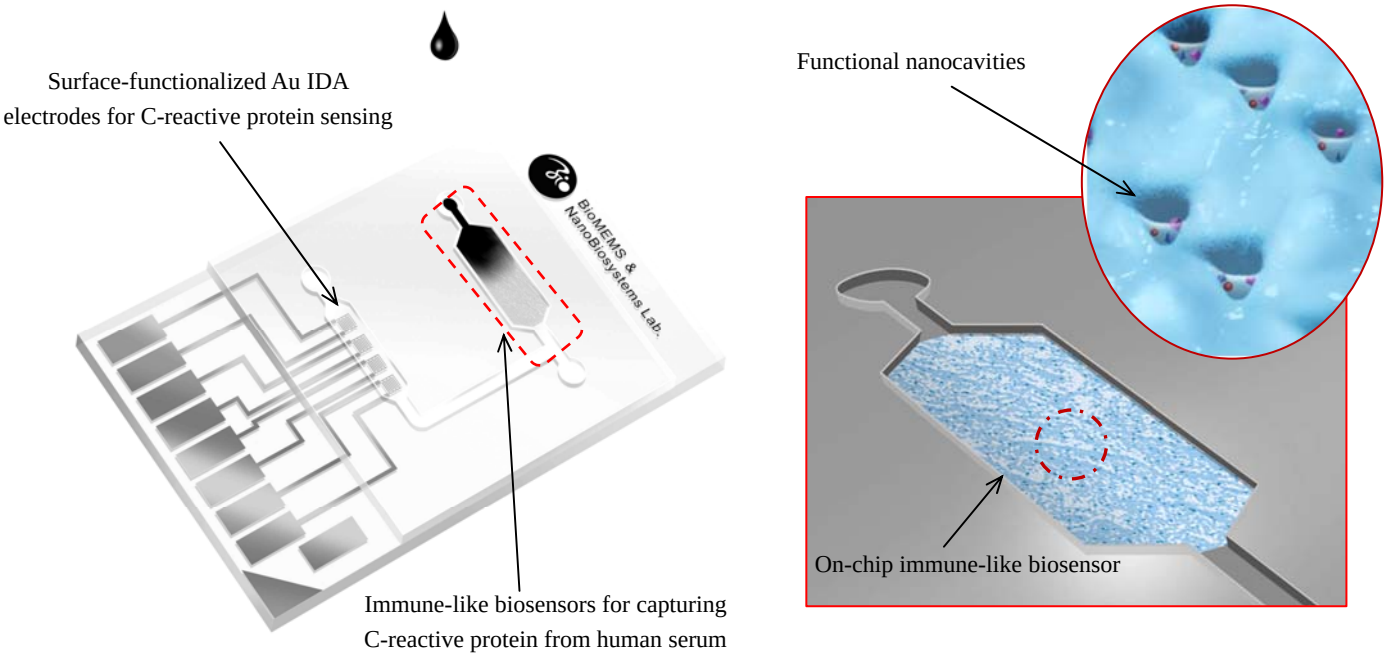


Figure 1. Schematic illustration of the developed electronic microfluidic biochips with on-chip immune-like biosensors for rapid detection of C-reactive protein in human serum.

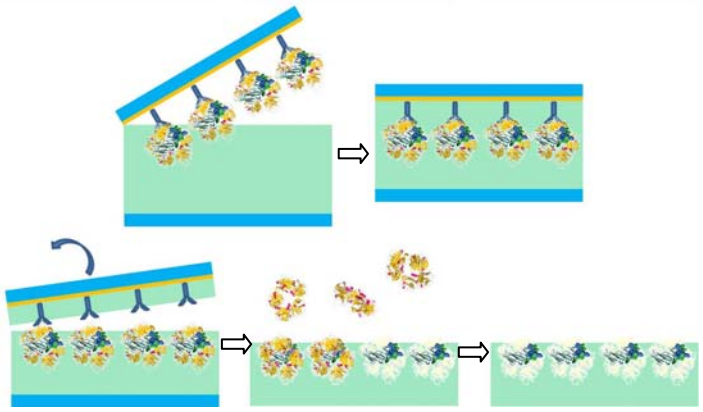


Figure 2. Fabrication process of the developed immune-like biosensors for CRP detection.

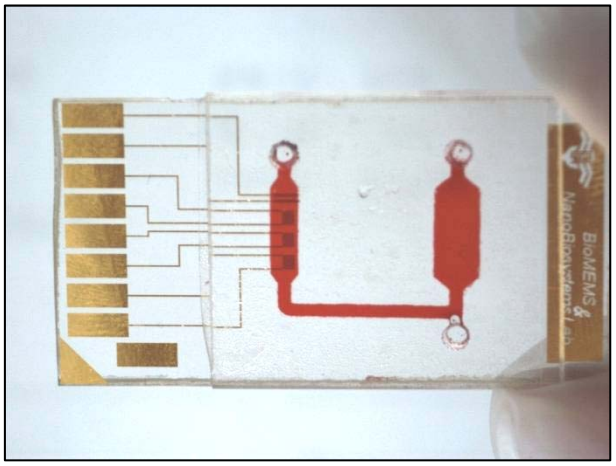


Figure 3. Photographs of the fabricated microfluidic biochip with on-chip immune-like biosensors.

EXPERIMENTAL RESULTS

Compared to non-protein imprinted polymers (NIP), the AFM images of the immune-like biosensors (PIP films) have nanocavities on the surfaces. According to the adhesion force measurements by antibody-coated AFM tips, the adhesion force caused by specific binding of immune-like biosensors was 29 nN, which is more than that of biological antibodies, as shown in Figure 4a. In addition, compared with non-imprinted polymers and non-aligned imprinted polymers, our developed highly-aligned imprinted polymers have the highest adhesion force caused by specific binding, as shown in Figure 4b. The specificity of PIP films was up to 450%. The adhesion force between PIP films and antibody-coated AFM tips showed no decrease at least for 3 months at room temperature. In the experiments, CRP samples were injected into the microfluidic biochips. After the separation by immune-like biosensors, SDS solvent was delivered to the separation microchamber films for C-reactive proteins extraction and then the SDS solvent with the extracted C-reactive proteins was delivered to the electrodes. The CRP concentrations in human serum were measured from the electrodes, as shown in Figure 5. The calibration curve was obtained simply by calculated discharging time constant from the dynamic voltage curves, as shown in Figure 6a. The linear range of the developed microfluidic microchip with immune-like biosensors is from 2 μg/ml to 200 μg/ml. In Figure 6b, another set of the known CRP samples were measured and calculated according to the above calibration curve. The response time of the developed biosensor is around 110 seconds. The electronic immune-like biosensors with the surface functionalized IDA electrodes for rapid detection of C-reactive protein presented in this work showed good performance in separation and sensing of C-reactive proteins.

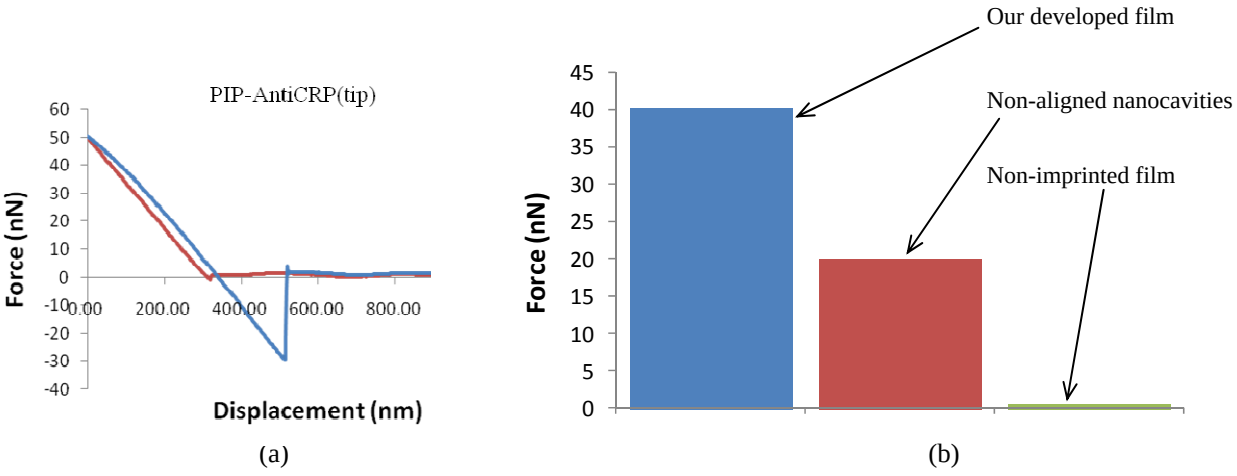


Figure 4. Characterization of the developed sensing films by AFM: (a) adhesion forces between our developed film and antibody and (b) comparisons of the adhesion forces caused by specific binding.

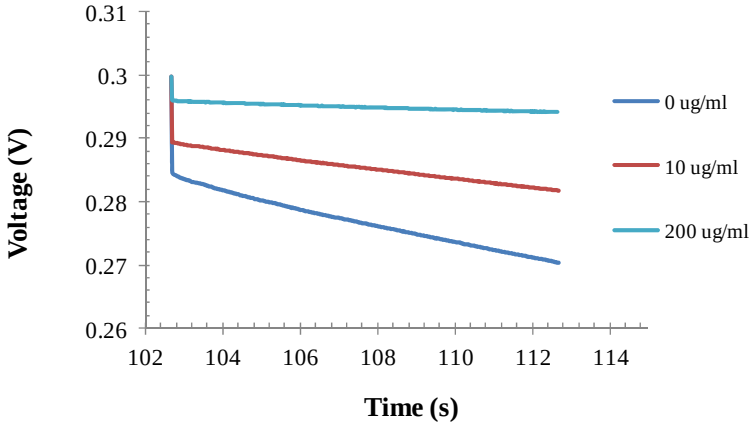
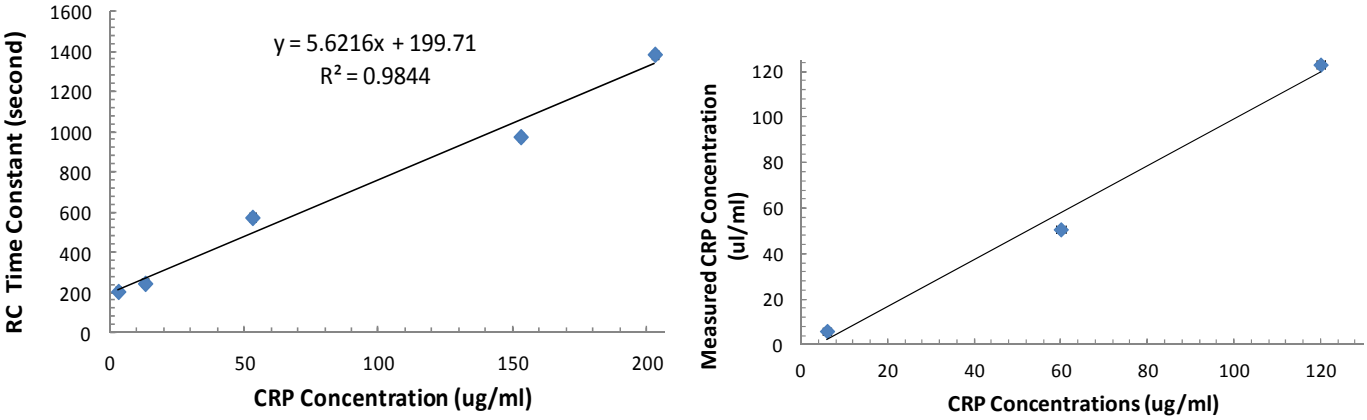


Figure 5. Dynamic voltage response of the IDA electrodes after the captured CRP was delivered to the electrodes from the immune-like biosensors.



(a) (b)

Figure 6. Measurement results of the developed biochips for rapid detection of CRP in human serum: (a) calibration curve and (b) the measured CRP concentrations according to the calibration curve.

CONCLUSIONS

In summary, novel electronic microfluidic biochips with on-chip immune-like biosensors for rapid detection of C-reactive protein in human serum has been developed and realized. The developed platforms with on-chip biomimetic biosensors have the advantages of fast detection response, ease of use, high sensitivity, and high selectivity.

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