ON-CHIP SURFACE PLASMON RESONANCE MEASUREMENT OF DISEASE MARKER PROTEIN AND SMALL METABOLITE COMBINED WITH IMMUNO AND ENZYMATIC REACTIONS

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ABSTRACT

We developed a PDMS based device for the simultaneous determination of the urinary diabetes marker, transferrin, and the small metabolite, creatinine, by the surface plasmon resonance (SPR) method. This SPR urine sensor can detect not only large molecules but also small substances by utilizing an enzymatic reaction. Transferrin and creatinine were detected by monitoring the antigen antibody immunoreaction and the redox state change from Os^{2+} to Os^{3+} followed by the reduction of hydrogen peroxide converted from creatinine, respectively. The detectable ranges were from 20 ng/mL to 10 μg/mL and 10 μM to 5 mM for transferrin and creatinine, respectively, and these meet the clinically required levels.

KEYWORDS: Immunosensor, surface plasmon resonance (SPR), creatinine, transferrin, osmium-poly(vinylpyridine)-wired horseradish peroxidase (Os-gel-HRP)

INTRODUCTION

Disease marker detection in urine samples is simple, rapid and non-invasive compared with detection in blood samples. However, the marker concentration must be corrected since the urine concentration changes greatly with water intake. Thus urine concentration has been standardized in terms of the creatinine that is also included in urine samples. Various methods have already been tried for detecting these two substances, but there is no satisfactory approach for detecting both substances with the same detector. Recently, the surface plasmon resonance (SPR) method has become attractive as a non-label and real time measurement technique for detecting disease markers. Although this method is very effective for large molecules, it is not sensitive for small molecules such as creatinine because of the small refractive index change. If we could detect a small substance by amplifying the SPR signal, we could obtain a size-independent SPR measurement system that would enable us to achieve simple point of care testing.

Here we show the simultaneous detection of both the small metabolite, creatinine, and the urinary diabetes marker, transferrin with the SPR method utilizing immuno- and enzymatic reactions in a microfluidic device.

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EXPERIMENTAL

Figure 1 shows a diagram and photographs of our integrated device. The circular gold film (4) is modified with capture antibody via protein A. We modified the other gold film (5) with Os(bpy)-HRP, and three enzymes in the upstream region of the film. Then two enzymes, ascorbic acid and creatine phosphorkinase were embedded in order to remove interference substances.

Figure 2 shows the creatinine and transferrin detection mechanisms, respectively. We used a portable SPR system to estimate the creatinine and transferrin from the SPR angle shifts caused by the enzymatic and immuno reactions on the gold film, respectively. We measured the creatinine concentration with the SPR method rather than the conventional electrochemical method, because the use of the same detection method reduces the overall system size.

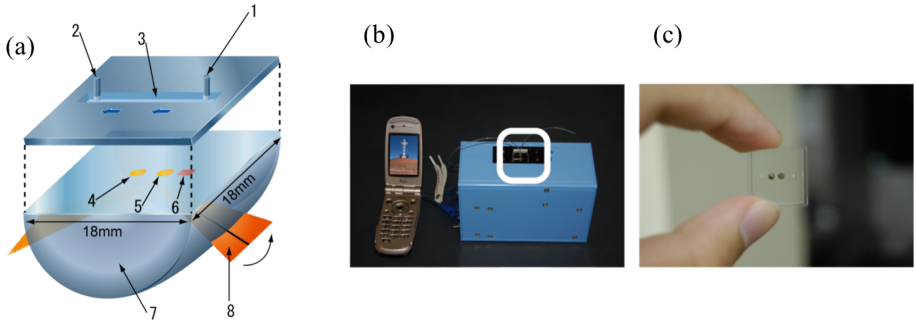

Figure 1. Diagram and photographs of our integrated device. (a) Glass plate with two gold thin film patterns and PDMS with a microchannel. (b) Photograph of portable SPR system. (c) Photograph of device. (1) Inlet, (2) Outlet, (3) microchannel (20 μm deep, 2 mm wide), (4) transferrin sensing gold surface modified with capture antibody via protein A, (5) creatinine sensing gold surface modified with Os-gel-HRP, (6) tri-enzyme layer modified to convert from creatinine to hydrogen peroxide, (7) coupling prism and (8) refracting light. Sample solution introduced in a suction mode.

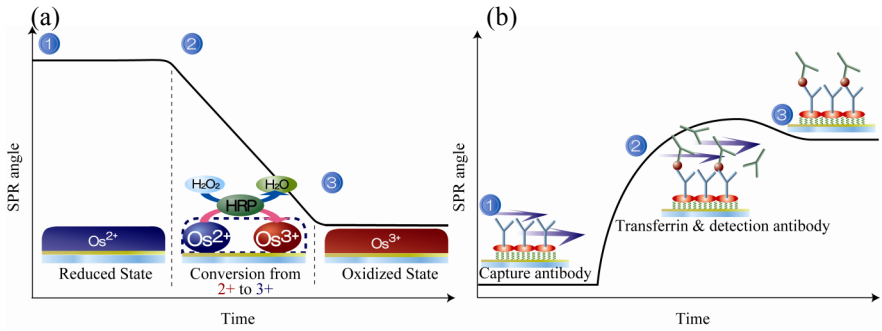

Figure 2. Diagram of (a) creatinine measurement using redox state change of osmium complex by hydrogen peroxide and (b) transferrin measurement by sandwich immunoassay.

RESULTS AND DISCUSSION

Figure 3 shows the variations in the SPR angles when we injected a sample solution containing 10 μg/mL transferrin and 1 mM creatinine for 13 min at a flow rate of 3 μL/min. The SPR angle was increased by the immuno-reaction and reduced by the enzymatic reaction. With our method, there is little crosstalk between the two sensing points because of BSA blocking. Figure 4 shows the calibration curves for transferrin and creatinine. The linear range of transferrin was from 100 ng/mL to 10 μg/mL with a detection limit of 20 ng/mL. Since the general cut off value for transferrin is around 800 ng/mL, this detection range satisfies the required clinical range. By contrast, the linear range for creatinine was 10 μM-5 mM. The creatinine calibration curve gradually saturated because of the dissolved oxygen concentration. Since the creatinine concentration in urine is around 2 to 30 mM, we could obtain the concentration accurately by diluting the urine sample several times.

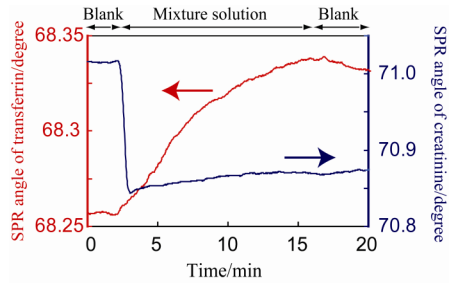

Figure 3. Variations in SPR angles when a mixture solution of anti transferrin antibody, and 1mM creatinine in 0.1 M phosphate buffer (pH7.4) was injected for 13 min. Flow rate was 3μ L/min.

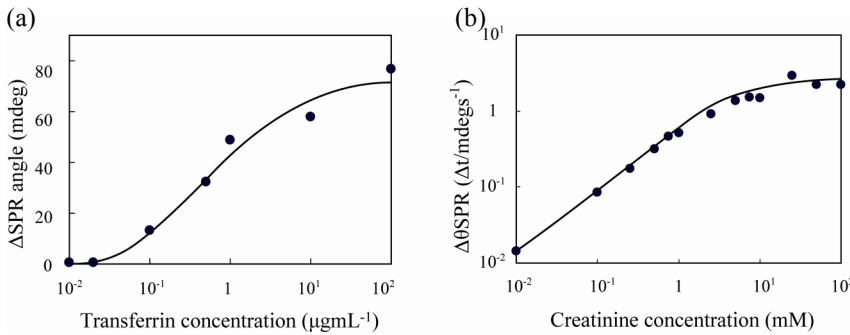

Figure 4. Calibration curves for (a) transferrin and (b) creatinine. The concentration range for transferrin and creatinine are from 10 ng/mL to 100 μg/mL and from 10 μM to 100 mM, respectively.

CONCLUSION

We were able to detect transferrin and creatinine simultaneously with a simple one-step injection using our sensor by integrating immuno- and enzyme sensing surfaces that could induce a sufficient SPR angle shift even for small molecules. This technique could be used to monitor various on-site biomarkers in urine by changing the capture antibodies. We will present the results of an real sample measurement.