IMMUNOASSAY DEVICE INTEGRATING PLASTIC FLOW-CHANNEL REACTOR AND RFID SENSOR CHIP

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ABSTRACT

An integrated immunoassay device (IID) for point-of-care testing (POCT)—comprising a plastic flow-channel reactor (FCR) and a radio frequency identification (RFID) sensor chip—was developed. The FCR provides a reaction field for immune and enzyme reactions. Sample/reagent solutions flow in the FCR by capillary force without any active mechanism like micro-pumps. The RFID-sensor chip monolithically integrates a photo sensor, a signal-processing circuit, and an RF wireless-communication circuit. Chemiluminescence (CL) enzyme is used as a detection marker of analyte protein, and CL was measured by RFID sensor. The IID achieves a lower detection limit of 1.3 ng/ml in hCG (human chorionic gonadotropin) concentration and quantitative measurement within 1 to 100 ng/ml.

KEYWORDS: POCT, Immunoassay, Chemiluminescence, Flow-channel, Sensor chip, RFID

INTRODUCTION

Optical-detection methods using fluorescence, bioluminescence, or chemiluminescence (CL) have been commonly used in the field of immunoassays because of their excellent sensitivity and specificity. However, an optical-detection system has yet to be applied to major commercial point-of-care testing (POCT) owing to difficulty in designing a low-cost, sensitive, and compact optical-detection mechanism for a miniaturized system [1]. Furthermore, as an alternative material to the conventional porous membrane of a reactor for POCT, (which has been widely used for immunochromatography but has insufficient reproducibility for quantitative measurement [1-3]), another material should be utilized. To address these issues, in the present study, an integrated immunoassay device (IID) consisting of two components, namely, a reaction chamber (i.e., a flow-channel reactor; FCR) and an sensor chip for CL detection, signal processing, and data transmission, was developed.

THEORY

The FCR enables a quantitative chemiluminescence assay with improved reproducibility by adopting controlled antibody immobilization and surface-blocking methods. A RFID sensor chip provides a simple and low-cost immunoassay by integrating a sensor, a signal-processing circuit, and a signal-transmitting interface. For the signal-processing interface, a wireless communication protocol conforms to the international standard (ISO/IEC15693); thus, a commercially available reader module can be used. This communication protocol makes it possible to implement a new kind of parallel measurement system. Thanks to an ID-number assigned to a chip, the reader module can identify each chip without any wiring or positioning; hence, parallel measurement of multiple samples—requiring no additional apparatus—becomes possible.

EXPERIMENTAL

The IID and its schematic cross-section are shown in Figs. 1 and 2. The FCR is made of a main substrate and an upper substrate by injection molding of cyclic olefin copolymer (COC). A primary antibody was immobilized on a test zone of the main substrate, and no antibody was on a control zone. Solution flow in the FCR was driven without using an active mechanism such as a pump. A solution was introduced at the inlet and flowed by capillary force and then by suction force of the flow-control channel and the absorbent. To maximize signal intensity and reproducibility, the flow rate was set at 28 µl/min by adjusting the length of the flow-control channel. As shown in Fig. 3, RFID sensor chips (2.5 by 2.5 mm in size), integrating a photosensor, a signal-processing circuit consisting of an amplifier/13-bit ADC, and an RF front-end with an antenna coil, were embedded just beneath the test and control zones. This arrangement of the sensor

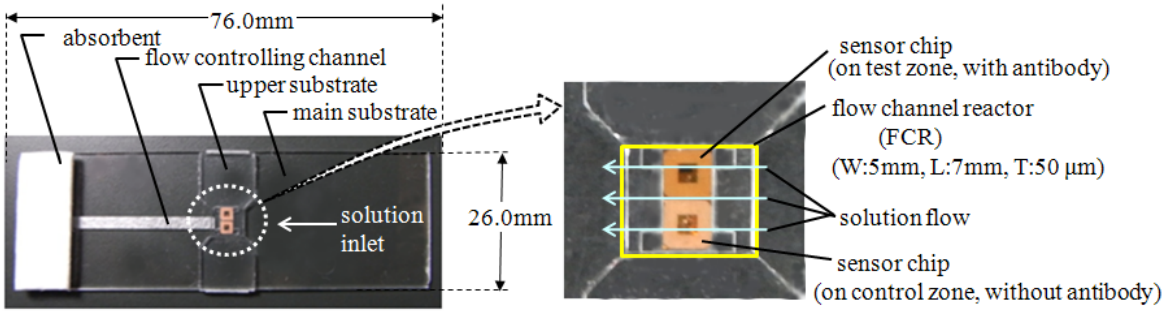

Figure 1: Photograph of integrated immunoassay device (IID) combining a plastic flow-channel reactor (FCR) and an RFID sensor chip

chip and FCR produces high optical coupling without need for a lens and alignment. Human chorionic gonadotropin (hCG) antigen was used as a test sample, and horseradish peroxidase (HRP) was adopted as an enzyme for CL. The sensor chips detect CL and transmit a CL signal to the reader in a wireless manner.

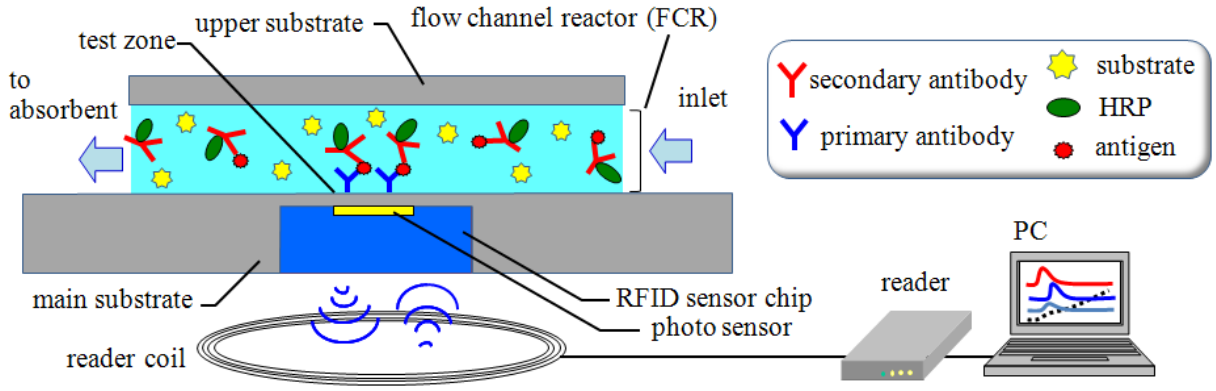

Figure 2. Schematic cross section of IID with reagents used by an immunoassay.

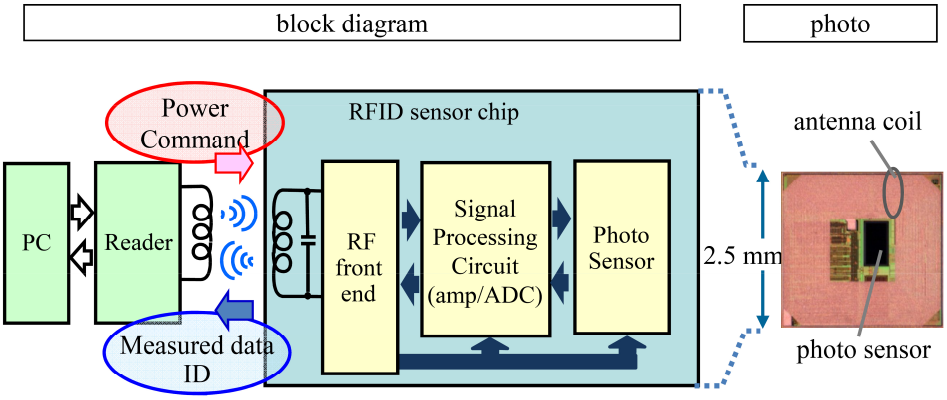

Figure 3: Photograph (right) and block diagram (left) of RFID sensor chip

Figure 4 shows a conceptual image of the immunoassay procedure with the IID. According to the anti-collision protocol [4] of the RFID sensor system, one reader communicates with multiple IIDs without any optical alignment or electrical-wire connection, as shown Fig. 4(b). This set up gives the IID flexibility and simplicity in parallel measurement of samples collected from multiple patients.

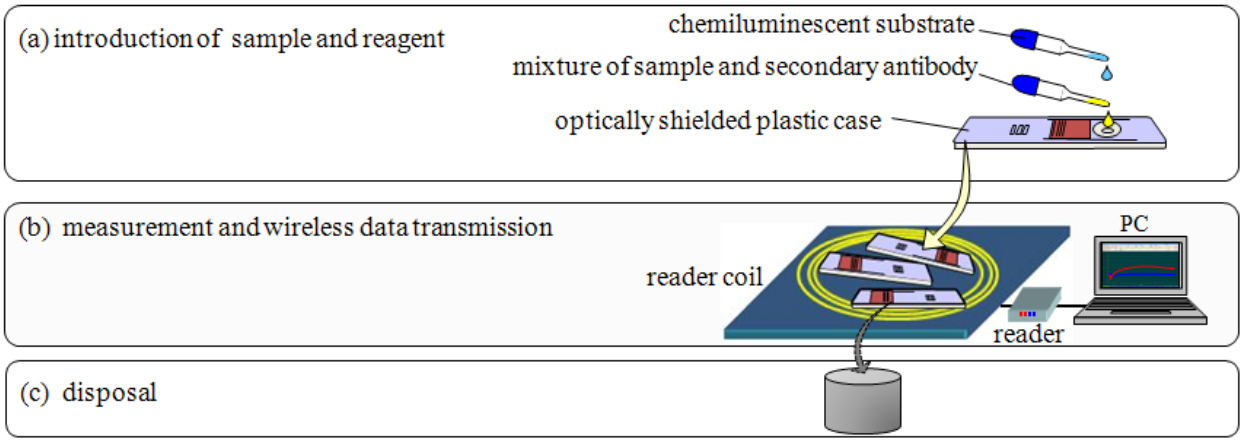

Figure 4: Conceptual schematic of measurement procedure.

RESULTS AND DISCUSSION

A calibration curve plotting signal intensity of the IID against antigen concentration is shown in Fig. 5. The lower limit of detection is 1.3 ng/ml, which is comparable to the best value by conventional immunochromatographic POCT measurement (i.e., 1–10 ng/ml). Moreover, quantitative measurement is demonstrated by the slope of the curve within the range of 1 to 100 ng/ml, where coefficient of variation (CV) is ≤ 31% (Table 1).

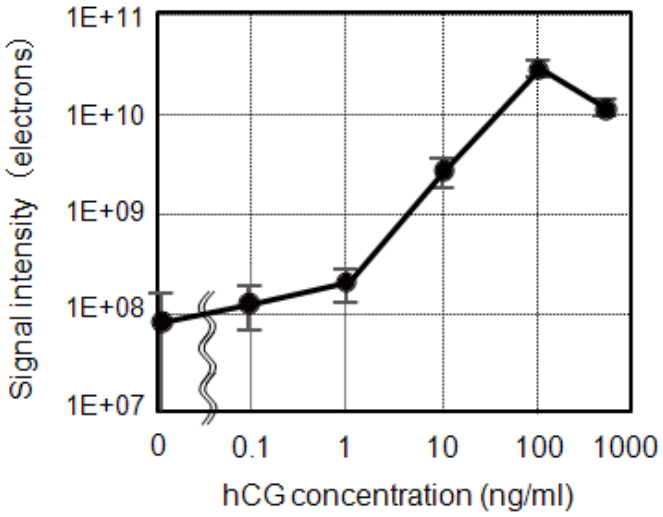

Figure 5: Calibration curve of hCG concentration (measured by IID) showing a detection limit of 1.3 ng/ml.

Table 1. Numerical values from measurement by IID. (N=5)

Antigen Conc. (ng/ml)	Signal (electrons)	SD (electrons)	CV (%)
500	1.2×10^{10}	1.9×10^{9}	16
100	2.8×10^{10}	4.7×10^{9}	17
10	3.2×10^{10}	9.9×10^{8}	31

CONCLUSION

An integrated immunoassay (IID) device for POCT comprising a plastic flow-channel reactor and a RFID-sensor chip was demonstrated. When hCG protein is used as a test analyte, the IID has a lower detection limit for hCG concentration of 1.3 ng/ml and can perform quantitative measurement within 1 to 100 ng/ml.

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