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Rapid immuno-analytical system physically integrated with lens-free CMOS image sensor for food-borne pathogens

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ABSTRACT

To realize an inexpensive, pocket-sized immunosensor system, a rapid test devise based on cross-flow immuno-chromatography was physically combined with a lens-free CMOS image sensor (CIS), which was then applied to the detection of the food-borne pathogen, *Salmonella typhimurium* (*S. typhimurium*). Two CISs, each retaining 1.3 mega pixel array, were mounted on a printed circuit board to fabricate a disposable sensing module, being connectable with a signal detection system. For the bacterial analysis, a cellulose membrane-based immunosensing platform, ELISA-on-a-chip (EOC), was employed, being integrated with the CIS module, and the antigen–antibody reaction sites were aligned with the respective sensor. In such sensor construction, the chemiluminescent signals produced from the EOC are transferred directly into the sensors and are converted to electric signals on the detector. The EOC-CIS integrated sensor was capable of detecting a traceable amount of the bacterium (4.22×10^3 CFU/mL), nearly comparable to that adopting a sophisticated detector such as cooled-charge-coupled device, while having greatly reduced dimensions and cost. Upon coupling with immuno-magnetic separation, the sensor showed an additional 67-fold enhancement in the detection limit. Furthermore, a real sample test was carried out for fish muscles inoculated with a sample of 3.3 CFU *S. typhimurium* per 10 g, which was able to be detected earlier than 6 h after the onset of pre-enrichment by culture.

1. Introduction

Immunoassay is an analytical method based on antigen–antibody binding reactions, which has been developed for the main purpose of quantifying a number of complex organic substances as analytes, and has been in use for more than half a century (Lequin, 2005). The reaction was initially carried out in the liquid phase, which was then adapted for use with solid matrices (Lequin, 2005). This transition was led by the demand for facilitating separation of the binding complexes from excess reagents used in the assay, where a 96-well microtiter plate has been used as the most popular platform (Butler, 2000). To trace the complexes, a number of signal generators have been employed and, among them, enzymes have been largely preferred, due to their ease of application (Butler, 2000). This format, called enzyme-linked immunosorbent assay (ELISA), is advantageous in handling multiple samples at the same time, as a step-by-step reaction procedure requires greater time, labor, expert knowledge, and laboratory instrumentation for signal measurement. Alternatively, a rapid, one-step assay version has been introduced, where immuno-chromatography is conducted on a porous membrane as the solid matrix, usually in combination with colloidal gold as a tracer (Preechakasedkit et al., 2011). A fluidic channel-based assay is also available as a relatively recent format in this category (Paek et al., 2007).

As a motivation of research, a high-sensitivity immunoassay has been in demand since the early detection of analyte may be essential in many fields for, for instances, in the control of disease and interception of hazard dissemination (Lim and Zhang, 2007). Furthermore, it should be widely applicable for use in various locations. We have indeed investigated a novel format of immunoassay that may simultaneously satisfy the needs by converting ELISA into a field test version based on immuno-chromatography, i.e., ELISA-on-a-chip (EOC; Cho et al., 2005, 2006). This may not only provide a sensitive result based on low analyte concentrations, but may also be utilized under non-laboratory conditions.

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Indeed, EOC has demonstrated competitive utilities in medical diagnosis (Cho et al., 2005, 2006), biodefense (Han et al., 2007), and food testing against pathogenic bacteria (Seo et al., 2009, 2010). The analytical systems currently in use, however, are still too bulky to carry in the hand, which is due to the adoption of a camera-type detector for the measurement of color or light signals from the assay (Mirasoli et al., 2012; Roda et al., 2011; Wongwilai et al., 2010). The minimal dimension of such as unit is determined by the focal distance which is required by the camera as well as the device volume itself.

In this study, we introduced a novel digital device for immunodiagnostics by incorporating a lens-free complementary metal–oxide–semiconductor (CMOS) image sensor (CIS) into the EOC, where a chemiluminescent signal is produced and measured in close proximity. Different to the use of a built-in CIS in camera, such an installation of the detector would offer several advantages, such as efficient photon transfer between two objects, as well as spatial saving leading to miniaturization. These have been attained by contacting two CISs fixed on a printed circuit board (PCB) directly onto each line for analyte and control of the signal generation pad of the EOC in an aligned manner. Such convergence technology for a compact immunosensor was assessed by applying it to the detection of a food-borne pathogen, *Salmonella typhimurium* (*S. typhimurium*; McClelland et al., 2001), comparing the performance with those of the conventional systems, and finally utilizing it for the analysis of real samples. In addition, we also introduced an immuno-magnetic separation (IMS) device with the function of enriching the bacterium prior to the analysis, further enhancing the sensitivity of the device for the analysis of bacterial contaminants.

2. Materials and methods

2.1. Materials

All reagents used in this study were of analytical grade and are listed in Supporting information.

2.2. Characterization of antibody to Salmonella species

Before testing the specificity of the anti-*Salmonella* rabbit antibody, it was chemically conjugated to horseradish peroxidase (HRP) as reported elsewhere (Nakane and Kawaoi, 1974). Each conjugate synthesized was mixed with the same volume of glycerol and was then stored at 4 °C prior to use. The antibody specificity was then tested by reacting it with different bacteria (*S. typhimurium*, *Salmonella enteritidis*, *Salmonella cholerasuis*, *Shigella sonnei*, *Vibrio parahaemolyticus*, and *Listeria monocytogenes*) immobilized on separate microwells. The binding was monitored by using either the enzyme conjugated with the primary antibody via cross-linking or a secondary antibody, goat anti-rabbit IgG, labeled with HRP. All experiments in this study were carried out in duplicate under the same conditions. Experimental details are not mentioned in the manuscript, but are presented in Supporting information.

2.3. Construction of EOC physically integrated with CIS

An immuno-strip, where antigen–antibody bindings occurred, was first prepared basically according to a previous report (Jeon et al., 2012). The strip consisted of four functional membrane pads, partially superimposed upon one another, for: (from the bottom) sample application, release of the detection antibody labeled with HRP, signal generation, and medium absorption. The signal generation pad was prepared by dispensing the anti-*Salmonella* capture antibody for the analyte line and goat anti-rabbit IgG for the control line on each pre-determined site. The four membrane pads were assembled on plastic film by using double-sided tape in the form of a 4 mm-wide membrane strip.

A two CISs (3.0×3.0 μm, 1.3 mega pixel array; refer to Supplementary Table 1 for detailed specifications)-mounted PCB was designed and fabricated as a module to integrate with EOC. The two sensors were located on the board to be aligned with the lines for analyte and control on the signal generation pad, respectively, when the module and EOC were combined together. The EOC used in this study was basically the same as the model introduced in a previous report (Jeon et al., 2012). The EOC plastic cartridge ($32 \times 76 \times 8$ mm) contained the immuno-strip in the vertical fluidic channel, and the CIS module was located close to the top of the signal generation pad. The light signal detected on CIS was converted to an electric signal through the use of a detector manufactured by this group (see Supplementary Fig. 1).

2.4. Analytical procedures

For signal detection on CIS, HRP was diluted and loaded onto the sensor of CIS module and the chemiluminescent substrate solution was immediately added. The catalytic light signal detected under dark conditions was stored as an image in a personal computer. For comparison, the colorimetric signal detection was also executed by using a substrate solution containing soluble tetramethylbenzidine dihydrochloride (TMB) in a microtiter plate. The color was measured at a maximum absorbance on a reader.

In analysis of *S. typhimurium*, immunoassay was carried out on EOC according to a previously described procedure (Seo et al., 2009, 2010). A standard sample was transferred into the application port and, after the vertical flow had occurred, the color signal was produced by adding insoluble TMB solution in a horizontal direction. The color image was captured and quantified in a numerical signal value (Jeon et al., 2012). For the chemiluminometric signal, the same protocol was employed except for the use of chemiluminogenic substrate in combination with a cooled-CCD as detector (Cho et al., 2009). After addition of the substrate solution, the EOC was placed within the detector system and the light signal was captured as an image 30 s later. Each analysis was carried out two times for the same sample and the mean values were plotted against the analyte concentration.

2.5. Construction of total analysis system for food-borne pathogen

To induce IMS, the anti-*Salmonella* antibody was first chemically coupled to tosyl-activated magnetic beads according to the protocol provided by the manufacturer. An IMS module enriching bacterial cells was also fabricated by modifying the previous model (Kim et al., 2012). To concentrate *S. typhimurium*, the sample was first combined with the immuno-magnetic beads and transferred into the IMS module. After a complete removal of aqueous medium, the cells bound onto the beads were dissociated and analyzed on EOC. Such analysis was duplicated, and the results were compared with those where the IMS stage was skipped.

The performance of the EOC–CIS biosensor system coupled with IMS was finally tested on flatfish which was purchased from a local market. The fish muscle was collected and treated according to the standard protocol (Seo et al., 2009, 2010). The prepared sample was artificially inoculated with *S. typhimurium* and then cultivated for pre-determined time periods. Each sample was concentrated by using the IMS module and subsequently analyzed on the EOC–CIS biosensor. The same experiment was carried out