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An immunochromatographic biosensor combined with a water-swellable polymer for automatic signal generation or amplification

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ABSTRACT

An immunochromatographic assay (ICA) strip is one of the most widely used platforms in the field of point-of-care biosensors for the detection of various analytes in a simple, fast, and inexpensive manner. Currently, several approaches for sequential reactions in ICA platforms have improved their usability, sensitivity, and versatility. In this study, a new, simple, and low-cost approach using automatic sequential-reaction ICA strip is described. The automatic switching of a reagent pad from separation to attachment to the test membrane was achieved using a water-swellable polymer. The reagent pad was dried with an enzyme substrate for signal generation or with signal-enhancing materials. The strip design and system operation were confirmed by the characterization of the raw materials and flow analysis. We demonstrated the operation of the proposed sensor by using various chemical reaction-based assays, including metal-ion amplification, enzyme-colorimetric reaction, and enzyme-catalyzed chemiluminescence. Furthermore, by employing C-reactive protein as a model, we successfully demonstrated that the new water-swellable polymer-based ICA sensor can be utilized to detect biologically relevant analytes in human serum.

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1. Introduction

Point-of-care (POC) diagnostic sensors have many advantages, including cost-effectiveness, simplicity, and rapid operation. Additionally, POC diagnostic testing increases the efficiency of the clinical process relative to central laboratory testing (Von Lode, 2005). Compared with central laboratory methods, POC diagnostic sensors have limitations in diagnostic accuracy owing to several drawbacks, including environmental effects, sample-handling errors, poor analytical performance, and post-test analysis (documentation) (Kost et al., 1999; Von Lode, 2005). In this regard, studies in the field of POC diagnostic sensors aim to overcome these drawbacks and have led to the design of microfluidic devices (Ko et al., 2008; Krejcova et al., 2014; Obubuafo et al., 2008) miniaturized total-analysis systems (Abrams et al., 2007; Chin et al., 2011; Qiu et al., 2009) and microfluidic paper-based analytical devices (He and Liu, 2013; Liu and Crooks, 2011; Martinez et al., 2007) as future alternatives (Tüdős et al., 2001). However, these technologies are not candidates for immediate commercialization owing to their relatively high costs, complex processing, and lack of verification (Su et al., 2015).

Among the commercialized POC diagnostic sensors, immunochromatographic assay (ICA) strip sensors are well-established immunoassay sensor platforms that are widely used beyond the clinical diagnostic field (Yetisen et al., 2013). Various types of ICAs have been used as diagnostic sensors for the detection of proteins (Cho and Paek, 2001; Oh et al., 2014), bacteria (Fang et al., 2014; Yu et al., 2011), viruses (Al-Yousif et al., 2002; Zhang et al., 2015), nucleic acids (Hu et al., 2013; Liu et al., 2013), and toxic molecules (Song et al., 2014; Wang et al., 2006). However, conventional ICA sensors using gold nanoparticles have drawbacks in the level of detection sensitivity and the quantitative assays that are required for precise diagnostic applications (Myers and Lee, 2008; Posthuma-Trumpie et al., 2009). To overcome these problems, various studies have refined signal-amplification or sensitive signal-generation methods, such as metal-ion signal amplification (Anfossi et al., 2013; Li et al., 2013), chemiluminescence assays (Kim et al., 2010; Mirasoli et al., 2012), and enzyme-colorimetric reactions (Kawde et al., 2010; Parolo et al., 2013; Samsonova et al., 2015) using ICA strip sensors. Additionally, several approaches attempted automatic reactions in a sensor using simple fluid technology or paper networks. For example, a plastic enzyme-linked immunosorbent

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assay (ELISA)-on-a-chip method employed a cross-flow of solution containing an enzyme substrate to detect cardiac troponin I (Cho et al., 2006). A two-dimensional paper-network (2DPN) format was also developed to enable multistep assays to be performed simply (Fu et al., 2012). Toley et al. (2015) reported a paper-microfluidic device that automatically controlled fluids using expandable elements. This device used water to expand the compressed sponges, which enabled the sponges to connect or disconnect by two pathways. Even though these methods increased the detection sensitivity of the ICA strip sensor, additional operational steps, such as multistep injection and/or washing, offset the advantages of the ICA sensor. We previously reported an automatic enzyme immunoassay based on ICA methods that utilized an asymmetric polysulfone membrane (ASPM) (Joung et al., 2014). In this sensor, the horizontal flow at the small-pore side was stronger than that at the large-pore side. Consequently, the ASPM delayed the release of the secondary reagents following the immune reaction. Moreover, this platform was adapted to an ICA strip format that did not require additional fluidic support. However, this sensor was limited by its use of the conjugation pad owing to the difficulty in separating conjugates and secondary reagents during sensor operation.

Here, we demonstrated a fully automated immunochromatographic sensor performing ELISA or second-signal enhancing immunoassays without an additional supporting apparatus. A key design element involved automatically switching the reagent pad from separation to attachment to the test membrane. The reagent pad was dried with an enzyme substrate for signal generation or with signal-enhancing materials. The automatic switching was accomplished by using a water-swellable polymer composed of polyvinyl alcohol. As shown in Fig. 1, the aqueous sample that was dropped onto the sample pad soaked and gradually swelled the water-swellable polymer. After a few minutes, the reagent pad below the water-swellable polymer came into contact with the test membrane, which was followed by the release of the secondary reagent from the reagent pad to the test membrane to generate or amplify the immunoassay signal. The feasibility of this strategy was demonstrated by the results obtained following the screening of various water-soluble tapes and the new ICA sensor in combination with a water-swellable polymer tape applied to various chemical reaction-based assays, such as metal-ion amplification, enzyme-colorimetric reactions, and enzyme-catalyzed chemiluminescence. Finally, the practical application of the developed biosensor coupled with a chemiluminescence assay was demonstrated by the detection of C-reactive protein (CRP) in human serum.

2. Materials and methods

2.1. Materials

CRP-free serum (90R-100) and surfactant 10G (95R-103) were purchased from Fitzgerald Industries International (Acton, MA,

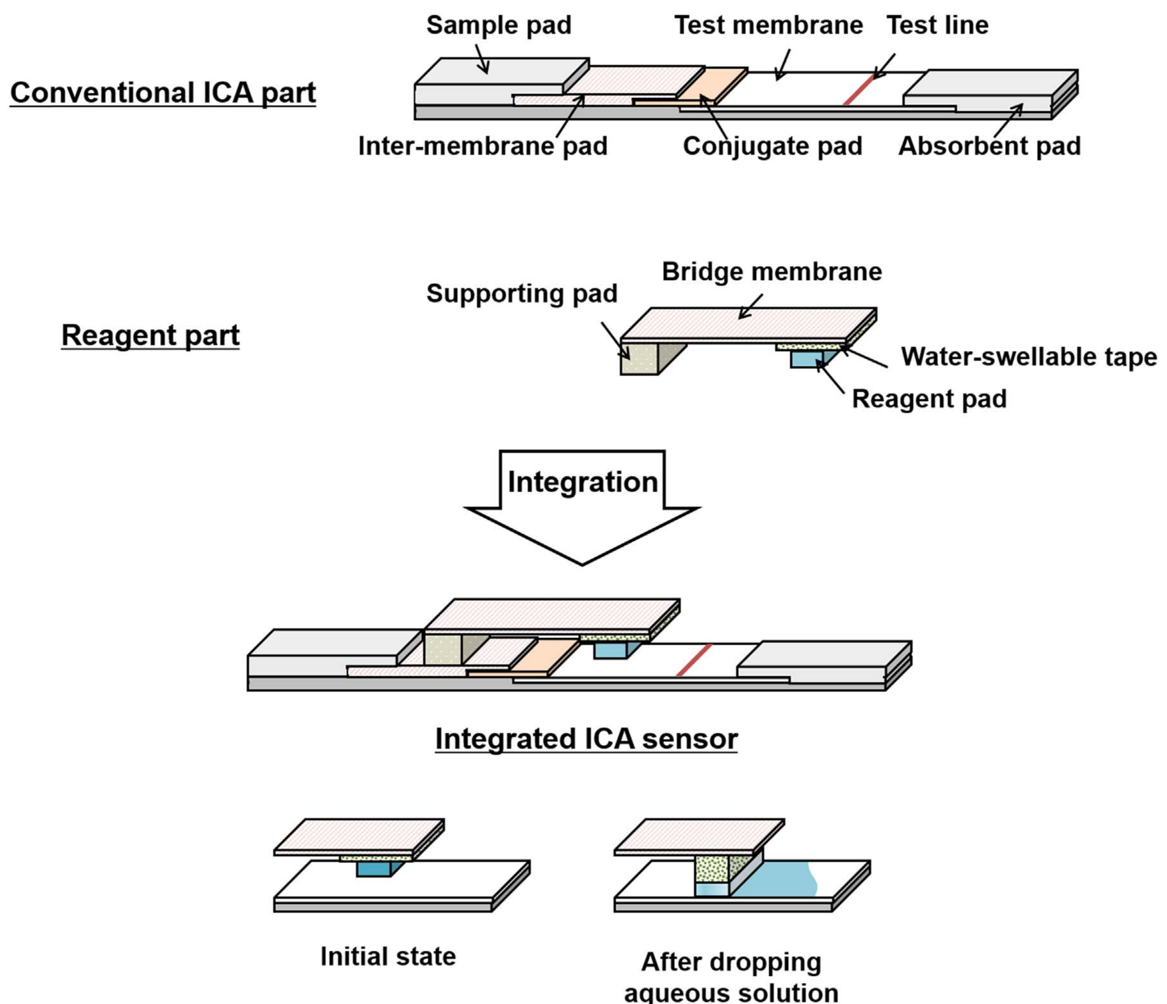

Fig. 1. Schematic illustrations of the suggested structure for the automatic sequential reactions. The water-swellable polymer was swelled and then switched the flow to the secondary reagent by aqueous solution.

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