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Immunoassay of paralytic shellfish toxins by moving magnetic particles in a stationary liquid-phase lab-on-a-chip

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ABSTRACT

In this study, we devised a stationary liquid-phase lab-on-a-chip (SLP LOC), which was operated by moving solid-phase magnetic particles in the stationary liquid phase. The SLP LOC consisted of a sample chamber to which a sample and reactants were added, a detection chamber containing enzyme substrate solution, and a narrow channel connecting the two chambers and filled with buffer. As a model system, competitive immunoassays of saxitoxin (STX), a paralytic shellfish toxin, were conducted in the SLP LOC using protein G-coupled magnetic particles (G-MPs) as the solid phase. Anti-STX antibodies, STX-horseradish peroxidase conjugate, G-MPs, and a STX sample were added to the sample chamber and reacted by shaking. While liquids were in the stationary state, G-MPs were transported from the sample chamber to the detection chamber by moving a magnet below the LOC. After incubation to allow the enzymatic reaction to occur, the absorbance of the detection chamber solution was found to be reciprocally related to the STX concentration of the sample. Thus, the SLP LOC may represent a novel, simple format for point-of-care testing applications of enzyme-linked immunosorbent assays by eliminating complicated liquid handling steps.

1. Introduction

Enzyme-linked immunosorbent assay (ELISA), which is based on the adsorption of an antigen or antibody to a solid-phase surface, is one of the most important diagnostic methods used today because of its high sensitivity and specificity. Typical ELISA methods involve multiple experimental steps, including addition of samples or reagents as well as removal of substances not bound to the solid-phase surface. However, these liquid-handling steps make the method laborious, time consuming, and unsuitable for point-of-care testing (POCT) applications. Although strip sensors based on the lateral flow immunoassay (LFI) method have been used for POCT, their applications are limited because of low sensitivity and reliability (Posthuma-Trumpie et al., 2009).

For more reliable POCT immunoassays, various types of lab-on-a-chip (LOC) systems have been developed (Bange et al., 2005; Ng et al., 2010; Lin et al., 2010). A LOC has an integrated microfluidics system on a single chip where all immunoassay steps can be carried out without complicated manipulations. LOCs have the potential to improve analytical performance by reducing the consumption of reagents, decreasing the analysis time, and increasing reliability and sensitivity. For commercialization of LOCs, however, device complexity must also be reduced to make systems that are more robust, cost effective, and user friendly.

Saxitoxin (STX) is a paralytic shellfish toxin (PST), a group of neurotoxins that are usually present in certain species of marine dinoflagellates and are responsible for the human illness known as paralytic shellfish poisoning (Isbister and Kiernan, 2005). Currently, the mouse bioassay is internationally accepted for monitoring of PSTs in shellfish (AOAC, 2005); however, this method faces increasing ethical concerns. As an alternative to the mouse bioassay method, methods based on high-performance liquid chromatography (HPLC)-fluorescence detection have been developed and approved by the Association of Analytical Communities (Lawrence et al., 2005). However, application of this method is limited because of time-consuming precolumn oxidation and sample preparation procedures. Although ELISAs and LFIs have also been developed for rapid detection of PSTs, these methods suffer from the typical drawbacks, as described above (Kasuga et al., 1996; Usleber et al., 2001; Garet et al., 2010; Laycock et al., 2009; Wong et al., 2010; Fraga et al., 2013, Szkola et al., 2013 and 2014; Kawatsu et al., 2014).

In this study, we devised a stationary liquid-phase (SLP) LOC for POCT application of immunoassays; for this SLP LOC, all procedures were carried out by moving solid-phase particles while the liquid phase was in the stationary state. Our method enabled the simple, rapid detection of STX and may have potential commercial applications.

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2. Materials and methods

2.1. Materials

Silicone elastomer base (Sylgard 184) and its curing agent were purchased from Dow Corning. Protein G-coupled magnetic particles (G-MPs) with a mean diameter of 1 μm were purchased from Pierce Biotechnology (Rockford, MD, USA). An analytical standard of STX was purchased from National Research Council Canada (Halifax, Canada). A STX ELISA kit was purchased from Beacon Analytical Systems (Saco, ME, USA). Blue Dextran 2000 was purchased from GE Healthcare (Uppsala, Sweden). Horseradish peroxidase (HRP)-conjugated anti-goat immunoglobulin G antibody (Ab-HRP) and HRP were purchased from Sigma (St. Louis, MO, USA). Other proteins, chemicals, and substrate solutions were purchased from Sigma unless otherwise stated.

2.2. Assay design and the STX model system

The SLP LOC was based on a simple design consisting of a sample chamber, detection chamber, and channel connecting the two chambers (Fig. 1). As a model system, STX immunoassays were conducted in the SLP LOC using MPs as the solid phase. For the competitive immunoassay of STX in the SLP LOC, anti-STX antibodies, STX-HRP, and G-MPs were added to the sample chamber, along with a sample containing STX. A magnet was placed under the LOC and moved from the sample chamber to the detection chamber filled with HRP substrate solution. Measurement of the absorbance of the detection chamber solution allowed for calculation of the STX concentration of the sample.

2.3. Preparation of the LOC

The SLP LOC was composed of a polydimethylsiloxane (PDMS) body, the top and bottom sides of which were sealed with glass plates of 1-mm thickness. Molds for the PDMS body were prepared with bronze, cut with a computerized numerical control milling machine, and polished by chromium plating. PDMS prepolymer (a mixture of 1:10 base polymer with curing agent) was poured onto the mold and incubated at 70 °C for 40–50 min. The resulting PDMS body had two holes for the chambers and a groove connecting the two holes at the bottom. The bottom side was sealed with a glass plate, yielding two chambers and a channel. The tops of the chambers were sealed with two separate glass covers, one for the sample chamber and one for the detection chamber, after buffers or reagents were added.

2.4. Preparation of the MP transport device

The MP transport device was prepared with a KDS-200 syringe pump (KD Scientific; Holliston, MA, USA), a vibrating motor, neodymium magnets, and a homemade LOC holder (Supplementary Fig. 1). The magnets were installed on the pusher block (moving part) of the syringe pump. The orientation of the magnets was adjusted to concentrate the MPs to a single spot. The LOC holder was made with acryl and installed on the lead screw holding block, which was a fixed part of the syringe pump. The vibrating motor was attached to the LOC holder to provide agitation to the LOCs.

2.5. Visible estimation of LOC efficiency

The LOC was filled with buffer B (1 mg/mL bovine serum albumin in phosphate-buffered saline [PBS]), and the detection chamber was sealed with a cover. After 20 μL of buffer B was removed from the sample chamber, G-MPs (20 μg in 10 μL) and Blue Dextran (20 μg in 10 μL) were added to the chamber. The sample chamber was also sealed with a cover. The LOCs were shaken for 30 min at 350 rpm with a CPS-350 shaker (JEIO Tech; Seoul, Korea) in order to mix the components in the sample chamber. While the LOC holder was agitated with the vibrating motor, the magnet was placed below the sample chamber for 1 min. The magnet was moved from the sample chamber to the detection chamber at a speed of 2 cm/min with a 10 s pause in the middle of the channel and finally placed below the detection chamber for 1 min.

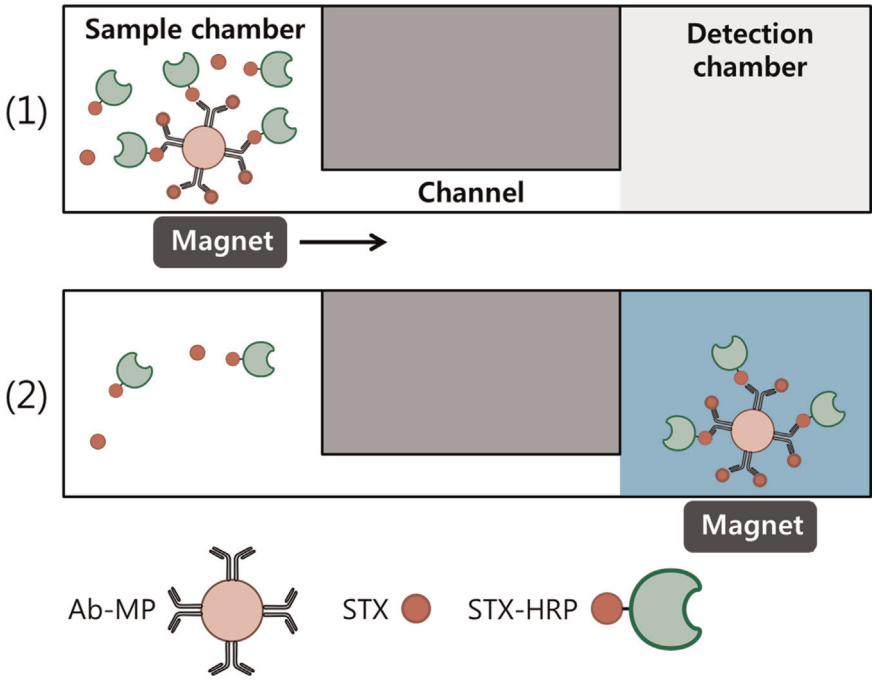

Fig. 1. Schematic representation of the principle of the SLP LOC.