DEVELOPMENT OF THREE-STEP CONSOLIDATING MICROCHIP FOR THERAPEUTIC DRUG MONITORING

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ABSTRACT

We developed easy-to-use microchip for therapeutic drug monitoring (TDM). Our assay platform consolidates three steps; preparation of reagents, quantitative liquid dispensing of sample and assay. The assay platform uses cloned enzyme donor immunoassay (CEDIA). The structure of the microchip permit liquid reagents and samples to be dispensed quantitatively [1]. Assay reagents were dispensed and lyophilized in the microchip. This microchip would permit one-step and rapid (5 min) assay, requiring only small volumes of reagent and sample (1.5 µl).

KEYWORDS: Therapeutic drug monitoring, Cloned enzyme donor immunoassay, Theophylline

INTRODUCTION

TDM is a method to administer adequate drug therapy. When therapeutic concentration range of the drug is narrow, it is significantly important to determine the drug concentration and to adjust the amount of dose. Recently, a chip-based CEDIA has been developed by using a well-type microchip for TDM, in which CEDIA reagent was lyophilized, and serum sample was easily assayed [2]. However, to assay whole blood, plasma separation step and quantitative dispensing step were individually required. Therefore our purpose is to develop microchip which consolidates four steps; plasma separation, preparation of reagents, quantitative dispensing and assay. As a first stage, here we consolidated three steps without plasma separation, and tested theophylline which is used for the treatment of asthma attacks, dissolved in phosphate buffered saline (PBS).

THEORY

CEDIA is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments: an enzyme donor (ED) and an enzyme acceptor (EA). The complementation of ED and EA forms an active enzyme. The covalent attachment of an analyte or a ligand to ED does not affect the ability of ED to associate with EA. Analyte-ED conjugate binding to an antibody does not associate with EA, and form an enzyme. An analyte present in a sample competes for binding to a limited number of antibody sites, making an analyte-ED conjugate available for enzyme formation. The amount of active enzyme formed is directly proportional to the analyte concentration in a sample. During the assay, the level of an enzyme with β-galactosidase activity can be determined spectrophotometrically by the rate of hydrolysis of a chromogenic substrate, chlorophenol red-β-galactopyranoside (CPRG), in a commercial CEDIA kit. Here we used fluorescent substrate, fluorescein di(β- D-galactopyranoside) (FDG).

The mixture of CEDIA reagents was prefilled in the microchip, and the scheme is illustrated in Figure 1(a). The mixture solution was injected into the liquid channel (Step 1). At this moment, the reagents entered into the reaction chambers and stopped at the entrance of narrow channels since the narrow channels acted as a kind of valve for aqueous liquid. Subsequently, air was injected into the channel to remove the excess reagents, and precise volumes of the reagents were left in the reaction chambers (Step 2). Following this, the microchip was lyophilized, and became ready-to-use (Step 3). The assay scheme is illustrated in Figure 1(b). Sample was injected into the channel (Step 1). Subsequently, air was injected to take precise volumes of the sample in the reaction chambers (Step 2). For the moment, lyophilized reagent was dissolved to the sample and CEDIA reaction occurred. Intensity of resultant fluorescence was measured by a fluorescence microscopy (Step 3).

EXPERIMENTAL

Two microchannels were fabricated on poly(dimethyl siloxane) (PDMS) by soft lithography. The schematic illustrations of the microchannels are shown in Figure 2(a). Air channel has 4 narrow channels for discharge of air which sizes are 5 µm width and 5 µm depth. Liquid channel has 4 reaction chambers which sizes are 100 µm width and 700 µm length. Two microchannels were attached to lap the reaction chambers over the narrow channels. This structure permits quantitative liquid dispensing [1]. Figure 2(b) shows the photograph of the microchip.

We used CEDIA theophylline II kit of Microgenics, which consists of Reagent 1 and 2. Reagent 1 was the combination of an EA powder and an antibody solution, and Reagent 2 was the combination of a powder of theophylline-ED conjugate and CPRG, and 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer. Reagent 2 of the CEDIA kit, but without CPRG was kindly provided by Microgenics. A mixture of the reagents was prepared by mixing 50 µl of Reagent 1, 48.6 µl of Reagent 2 without CPRG and 1.35 µl of 0.1 M FDG in dimethyl sulfoxide (DMSO).

Before injection of the mixture of the reagent, the Liquid channel was exposed to air plasma and attached to the Air channel. The Liquid channel was coated with 10% bovine serum albumin. The mixture of the reagents was injected. Subsequently, air was injected by conventional syringe. After dispensing of the reagents, the microchip was lyophilized for one day.

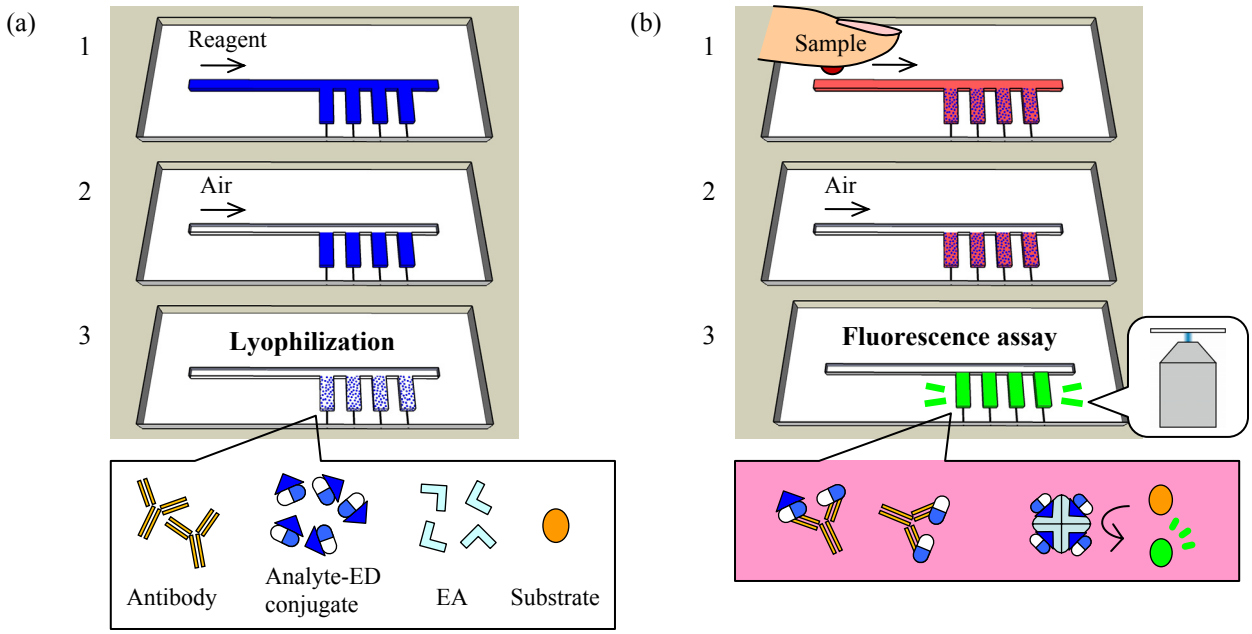

Figure 1: (a) Schematic illustration of prefilling CEDIA reagent in the microchip; reagent injection (Step 1), air injection (Step 2) and lyophilization (Step 3). (b) Schematic illustration of assay; sample injection (Step 1), air injection (Step 2) and fluorescence assay (Step 3).

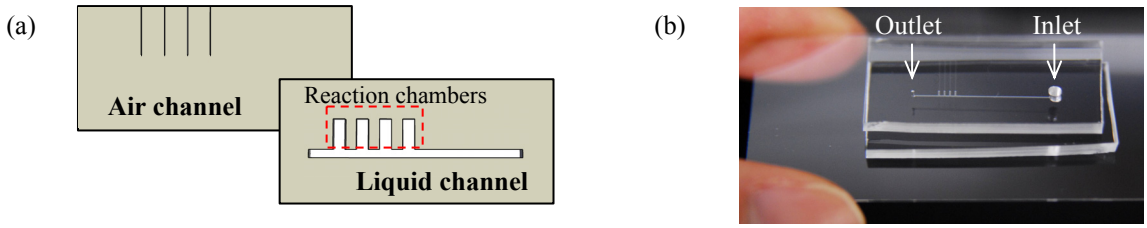

Figure 2: (a) Schematic illustrations of two channels; Air channel and Liquid channel. (b) Photograph of the microchip.

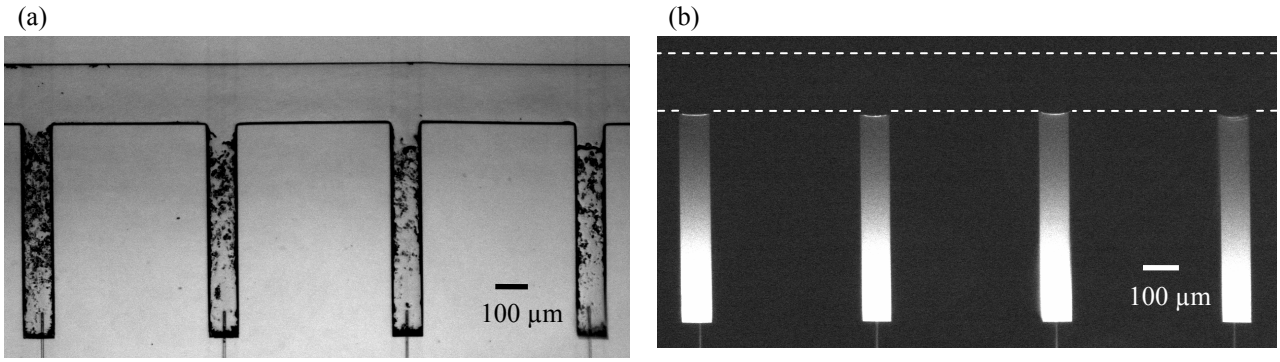

Figure 3: (a) Photograph of the reaction chambers containing lyophilized reagents. (b) Fluorescence image of the reaction chambers 5 min after sample injection.

The concentration of the theophylline solution were 0, 10, 20, 40 µg/ml. Each theophylline solution were prepared by adding theophylline to PBS. The solution were injected into the microchip in which the mixture of the reagents were lyophilized. Subsequently, air was injected. The fluorescence intensities of the reaction chambers were measured by using fluorescence microscope and CCD camera.

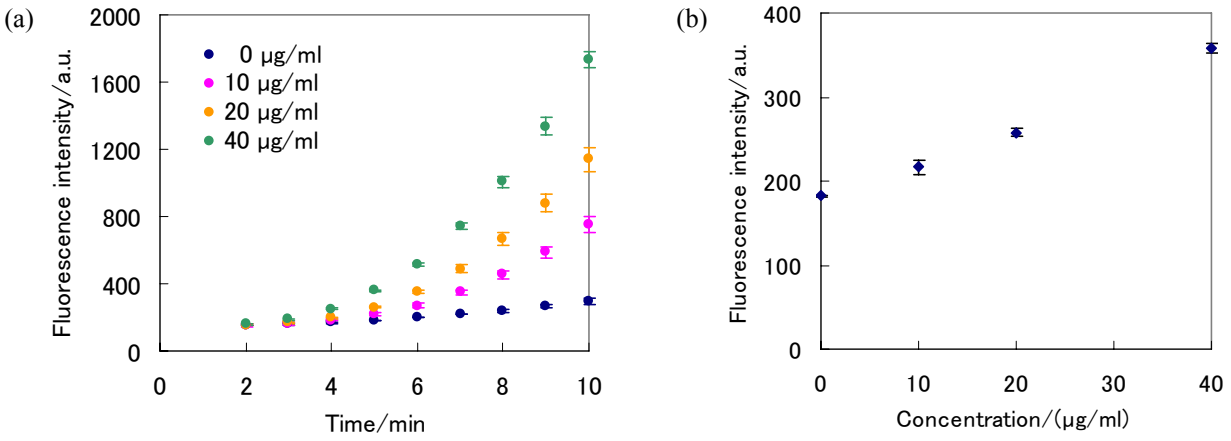

Figure 4:(a) The time course of fluorescence intensity with various concentration (0, 10, 20 and 40 μg/ml) theophylline solution. (b) The calibration curve plotting fluorescence intensity against the theophylline concentration at 5min after sample injection.

RESULTS AND DISCUSSION

Figure 3(a) shows the photograph of the microchip in which the mixture of the reagents was lyophilized. This result shows the lyophilized reagents exist only the reaction chambers. Figure 3(b) shows a fluorescence image of 5 min after theophylline solution (40 μg/ml) injection. It indicates the reaction of CEDIA correctly occurred and fluorescence signals were detected.

The fluorescence intensities of theophylline solutions (0, 10, 20 and 40 μg/ml) were measured by the minute, and the results are shown in Figure 4(a). This figure indicates fluorescence intensities with each concentration of theophylline increased over time. As shown Figure 4(b), the values of fluorescence intensity were correlated with the concentration of theophylline around the range of therapeutic drug concentration (10-20 μg/ml) at 5 minutes after theophylline solutions injection. Therefore it was suggested our method can determine the drug concentrations in blood in 5 minutes.

CONCLUSION

We developed a novel assay platform for TDM which consolidates three steps; preparation of reagents, quantitative liquid dispensing of sample and assay. This platform provides rapid analysis (5 min), it is easy-to-use, and it uses small volumes of the sample and reagent (1.5 μl each). As a next stage, we plan to consolidate plasma separation step, and achieve one-step assay of whole blood. We believe that it has a great potential for TDM.

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