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Fabrication and packaging of a mass-producible capillary-assembled microchip for simple and multiplexed bioassay

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ABSTRACT

We describe the fabrication and simple packaging of a mass-producible analytical microdevice, the third-generation capillary-assembled microchip (CAs-CHIP-3G), capable of single-step and multiplexed bioassay. The packaged CAs-CHIP format is an assembly of a laminated-dry reagent-release capillary (dRRC) array (CAs-CHIP-3G), paper pad, and vinyl tape (electrical tape) that acts as biosensor array, absorbent of excess sample solution, and adhesive for forming sample introduction microchannel, respectively. Sample introduction is carried out by simply piercing the vinyl tape with syringe and subsequent manual injection. No external pump is necessary since the fluid actuation for sample introduction into the dRRC array and subsequent removal of excess sample solution by paper pad are both spontaneously occurred by capillary action. Evaporation of sample solution is prevented by sealing both ends of dRRC array with oil allowing long time incubation for enzyme reaction. To demonstrate the potential application for simple and multiplexed bioassay, multiplexed glycosidase enzymes (α- and β-galactosidase, α-glucosidase, α-mannosidase) assay are carried out as an example.

1. Introduction

Development of an analytical tool for multiplexed bioassay is critical to understanding and monitoring of various biological events. Implementing these bioassays in microfluidic format is attractive since minimal consumption of expensive reagents/valuable samples and automation could be achieved [1–5]. Simple-to-use microfluidic biosensors are packaged in various formats such as centrifugal microfluidics/lab-on-a-CD (Lab CD) [6,7], SlipChip [8,9], volumetric bar-chart chip (V-Chip) [10], slip-based paper analytical device (SlipPAD) [11], and other microfluidic paper-based analytical device (μPAD) [12–14]. In these formats, single or multiple operational step/s and simultaneous assays of various analytes such as antigen [15,16], enzyme [17,18], or DNA [19,20] were implemented. In particular, microfluidic enzyme biosensor is a promising bioanalytical tool for clinical diagnostics and drug screening. Chen et al. have demonstrated multiplexed protease activity assay and its application to endometriosis using droplet-based microfluidics [21]. On the other hand, Sista et al. have reported the multiplexed enzyme assay for lysosomal storage disease screening in newborns using the digital microfluidics by analyzing various glycosidase enzymes [22]. These microfluidic multiplexed enzyme assays have shown their potential as effective analytical tools, however, they require external pumping system or droplet actuation control system. In order to meet the requirement of biological research in common research laboratory, easy-to-fabricate and -use, inexpensive, and disposable device capable of multiplexed bioassay including enzyme activity assay is necessary.

Alternatively, the capillary-assembled microchip (CAs-CHIP) is a microfluidic platform developed by our group wherein various functional glass capillaries are integrated into a single microfluidic device by embedding them into polydimethylsiloxane (PDMS) microchannels to allow for the development of a flexible multiparametric analytical microdevice [23,24]. We have developed various glass capillary biosensors to be integrated into CAs-CHIP, to measure various analytes such as proteins [25–28], ions [29,30], enzymes [30,31], and metabolites [32,33] in a sample solution. By using a single CAs-CHIP, various multiplexed bioassays have been successfully demonstrated by choosing various combinations of capillary biosensors [29–31,34,35].

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Among many capillary biosensors developed, dry reagent-release capillary (dRRC) which allows spontaneous dissolution of analytical reagents and selective reaction to give fluorescence response by sample introduction via capillary action, played an important role for the simplicity in capillary biosensor preparation and biosensing [30]. However, both the first (CAs-CHIP-1G) [23,30,31] and second (CAs-CHIIP-2G) [35] generations of CAs-CHIP requires the meticulous one-by-one capillary embedding onto a PDMS microchannel. This issue could limit its ease of fabrication, usability, and diminish the potential for mass production.

Here, we describe a simple and potentially mass-producible fabrication procedure of the third-generation capillary-assembled microchip (CAs-CHIP-3G), and its packaging procedure to be an easy-to-use and inexpensive device. Evaluation of sample introduction and application to multiplexed bioassays using packaged CAs-CHIP-3G are carried out.

2. Experimental

2.1. Reagents and materials

The poly(vinyl chloride-*co*-vinyl acetate-*co*-vinyl alcohol) (PVC copolymer), bovine serum albumin (BSA), glycosidase enzymes (α- and β-galactosidase (α- and β-gal), α-glucosidase (α-glu), and α-mannosidase(α-man)) and their substrates; 4-methylumbelliferyl-α-D-galactopyranoside (4-MU-α-gal), 4-methylumbelliferyl-β-D-galactopyranoside (4-MU-β-gal), 4-methylumbelliferyl-α-D-glucopyranoside (4-MU-α-glu), and 4-methylumbelliferyl-α-D-mannopyranoside (4-MU-α-man) were all obtained from Sigma (St. Louis, MO, USA). Polyethylene glycol (*Mn*: 20,000) (PEG-20000), tetrahydrofuran (THF), tris(hydroxymethyl)aminomethane (Tris), magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$), potassium chloride (KCl) and dimethylsulfoxide were purchased from Wako (Osaka, Japan). 4-(2-Hydroxyethyl)-2-piperazine-1-ethanesulfonic acid (HEPES) and dioctyl phthalate were acquired from Dojindo (Kumamoto, Japan) and TCI (Tokyo, Japan), respectively. The silicone oil was purchased from Shin-Etsu (Tokyo, Japan). All reagents were used without further purification. Distilled and deionized water used had resistivity values of more than $1.8 \times 10^7\ \Omega$ cm at 25 °C.

Square capillaries having 300-μm outer widths (flat-to-flat) and 100-μm inner widths were purchased from Polymicro (Phoenix, AZ, USA). The polyimide coating of these capillaries was removed by heating before use. The glass-cutting knife and 100-μL syringe were acquired from Sigma (MO, USA) and Hamilton (NV, USA), respectively. A paper pad (filter paper), transparent vinyl tape, and color vinyl tape (black) were obtained from Toyo Roshi Kaisha, Ltd. (Tokyo, Japan), Askul Corporation (Tokyo, Japan), and Nichiban Co. Ltd. (Tokyo, Japan), respectively.

2.2. Fabrication and packaging of CAs-CHIP-3G

The CAs-CHIP-3G for testing sample introduction or multiplexed enzyme assay was prepared by arraying twelve square glass capillaries (or dry reagent-release capillaries (dRRC)) and glass plates having 300 μm thickness and 2 mm width, in which the glass plates were used for ease of handling. These aligned capillaries and glass plates were adhered side-by-side by plasticized PVC. It was carried out by applying appropriate amount of THF solution of plasticized PVC composed of 400 mg of PVC copolymer, 800 μL of dioctyl phthalate, and 1.5 mL of THF on the aligned-capillary array, then, immediately blow-dried for a few minutes to evaporate the THF solvent (Fig. 1(A)). This process was repeated on the other side of the capillary array and sealed with vinyl tape both sides. Finally, the long glass capillary array sandwiched by vinyl tapes was further laminated without heat by using a pouch laminator (DS320P, GBC; IL, USA). From a 10-cm laminated capillary array, a 1-cm long CAs-CHIP-3G was cut using a glass-cutting knife.

The final image of analytical device (Packaged CAs-CHIP-3G) is shown in Fig. 1(B). A piece of filter paper (1 cm × 0.8 cm) was placed on the acrylic plate with black vinyl tape, on which the CAs-CHIP-3G was further layered and sealed by transparent tape.

To introduce the sample solution, the transparent vinyl tape was pierced at two points by using a micro syringe to prepare the inlet and air vent holes, whereupon 15 μL of sample solution was introduced. After filling all the capillaries and absorbing the excess sample solution by a paper pad, silicone oil (~50 μL) was introduced until sealing of both ends of the capillary array was visually confirmed (Fig. 1(C)).

For the sample introduction experiment, CAs-CHIP-3G was prepared by transparent vinyl tapes to visually observe the introduction process of blue dye (0.1% bromothymol blue) solution into capillary array.

2.3. Fabrication of packaged-CAs-CHIP-3G for multiplexed glycosidase enzyme bioassay

CAs-CHIP-3G for glycosidase enzyme assay was prepared with capillary biosensors (dry reagent-release capillary: dRRC) for glycosidase enzymes. They were prepared by immobilizing the enzyme substrates such as 4-MU-α-gal, 4-MU-β-gal, 4-MU-α-glu and 4-MU-α-man with PEG 20000 as soluble coatings. Coating was carried out by introducing the cocktail solution (Concentrations: 0.59 mM substrate with 0.5 mM PEG-20000) into the clean 10-cm long square glass capillary and then vacuum drying the capillary for about 8 h. The buffer used for all the experiments was 100 mM Tris containing 2 mM KCl, 0.1 mM $MgCl_2$, 0.1% BSA and 0.05% Tween-20 at pH 7.8.

For the multiplexed glycosidase enzyme bioassays using the CAs-CHIP-3G, three replicates of dRRCs dedicated for each enzyme were arrayed to finally package a 4-plexed CAs-CHIP-3G (twelve capillaries). Selectivity test was conducted by introducing a mixture solution containing the four different glycosidase enzymes or a solution containing one glycosidase enzyme into the packaged CAs-CHIP-3G (0.68 U/mL α-gal, 0.065 U/mL β-gal, 2.3 U/mL α-glu and 1.2 U/mL α-man). After sealing with oil, the packaged CAs-CHIP-3G was incubated for 30 min at room temperature.

Fluorescence was detected by a fluorescence microscope and a cooled CCD color camera (VB-7000, VB-7010, VB-L10, Keyence; Osaka, Japan) using 387/28-nm excitation filter and a 430-nm emission filter.

3. Results and discussion

3.1. Fabrication and sample introduction of packaged CAs-CHIP-3G microanalytical device

Figs. 1(A) and (B) show the general idea of the fabrication and packaging of the CAs-CHIP-3G microanalytical device, which is simple and the materials used are readily available and inexpensive.

Actual fabrication procedure is shown in Fig. 2. Aligning square glass capillaries was quite simple and easy, since the cross-sectional shape of capillary was square. After temporary fixing the inlet and outlet of the aligned capillary array by Scotch tape, appropriate amount of THF solution of plasticized PVC was applied and extend it along the capillary length direction with soft PDMS plate. In a preliminary experiment, capillary array was directly laminated with vinyl tape without applying plasticized PVC. However, since the outside structure of the square glass capillary is not perfectly square, after lamination of the arrayed capillary, gaps were formed