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The dual role of deposited microbead plug (DMBP): A blood filter and a conjugate reagent carrier toward point-of-care microfluidic immunoassay

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ABSTRACT

To set up a point-of-care whole-blood immunoassay system, sample preparation and on-chip storage of conjugate reagents are indispensable functional units. Here, we merge these functions into a deposited microbead plug (DMBP) to simultaneously play the roles of a blood filter and a conjugate reagent carrier. The DMBP was easily fabricated by the use of natural deposition of beads without the need of weirs. Conjugate reagents (FITC labeled antibodies used here) were incorporated into the DMBP during the assembly of the DMBP. To demonstrate the ability of the DMBP, we constructed a DMBP-based microfluidic chip and used it for the detection of human IgG (hIgG). The DMBP enabled to remove blood cells from whole blood and provide the pure plasma for the downstream on-chip immunoreactions. The release of reconstituted FITC labeled antibodies from the DMBP was controlled in a passive fashion. Dry FITC labeled antibodies retained at least 81% of their activity after 60 days of storage at the room temperature. The DMBP presented here makes an important step towards the development of the self-contained, integrated, sample-to-answer microfluidic chips for point-of-care diagnostics.

1. Introduction

Immunoassay based on the specificity of the antibody–antigen reaction is widely used in basic research and clinical diagnostics. Conventional immunoassay is usually done by technicians in the laboratory, involving laborious sample preparation process, multiple pipetting reagent steps, and time-consuming immunoreactions and detection. Point-of-care based immunoassay puts forward the new concept which promises to provide a convenient and immediate test to the untrained user at bedside. To realize this concept, a device which uses small raw samples taken directly from the patient and enables to integrate all the handling steps from sample to result with minimal user intervention would be preferred [1]. On-chip blood separation and reagent pre-storage are indispensable components in point-of-care devices.

Microfluidics with distinct advantages including small sample volumes, rapid results, and integration of multiple laboratory functions onto a single chip become an ideal platform for the point-of-care assays. A number of microfluidic devices for blood separation has been developed using various principles such as filtration [2–11], acoustic force [12,13], centrifugation [14,15], and biomimetics [16–19]. Recently, few cases have fulfilled the on-chip integration of blood separation with downstream analysis. For example, Fan et al. presented a biomarker detection chip on which blood separation based on the Zweifach-Fung effect was integrated [20]. Dimov et al. reported a blood analysis system that utilized blood cell sedimentation to realize on-chip blood separation [21]. Lee et al. performed whole blood immunoassay on a centrifugal microfluidic platform, which extracted plasma from whole blood using centrifugal force generated by the rotation of CD-like chip [22].

Currently, according to the state of reagents, the studies of reagent pre-storage on a microfluidic chip can be divided into two categories: liquid [23,24] and dry power [25,26]. Pre-storage of liquid in glass ampoules provided a method to prevent evaporation of liquid since polymer chip materials, especially PDMS, are permeable to vapor [27]. The glass ampoules filled with the various liquid reagents were placed on a centrifugal microfluidic platform. The on-demand release was realized by the regulation of rotational speed to crush the glass ampoules. Alternative method was to create discrete reagent droplets with the use of immiscible phases [28,29]. Compare with liquid reagents, dry reagents would have a longer life time especially in extreme environmental conditions. Stevens et al. demonstrated on-chip microfluidic immunoassay on the polymer card into which a fibrous pad containing dry labeling antibody was incorporated [30]. Hitzbleck et al. presented a reagent integrator where reagents were loaded by an inkjet spotter and dried in air. This reagent integrator enabled to control the release of reagents [31].

Abbreviations: Deposited microbead plug, (DMBP); Human IgG, (hIgG); Bovine serum albumin, (BSA); Goat anti-human IgG, (g-hIgG); Phosphate buffered saline, (PBS); Charge-coupled device, (CCD); Standard deviation, (SD)

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Although some examples fulfill reagent storage or blood separation on the microfluidic platform, most of them still require laborious manual handling or expensive peripheral equipment. Moreover, the integration of these components could dramatically increase complexity and cost of chip fabrication. So, the realization of both functions on a passive microfluidic chip with a simple, low-cost, and non-instrumented method is still a challenge.

In this study, we present the deposited microbead plug (DMBP) combining two functions which not only provides the on-chip separation of whole blood, but also allows the long-term preservation of all required reagents. Based on the previous work utilizing the DMBP to separate blood [32], we further integrate the function of the on-chip reagent storage into the DMBP. Conjugate reagents (FITC labeled antibodies used here) were incorporated into the DMBP during the assembly of the DMBP. The internal porous structures of the DMBP can block blood cells while allow blood plasma to penetrate. The filtered plasma reconstituted dry conjugate reagents pre-stored in the DMBP during it passed through the internal interstices of the DMBP. Capillary forces are used to drive fluids during the assay. The operation for users only needs to drop blood samples at the inlet. The release profile of conjugate reagents was characterized in detail. Dry conjugate reagents stored in the DMBP retained at least 81% of their activities after 60 days of storage at the room temperature. Merging both functions into the DMBP enables to simplify cumbersome immunoassay operations and make the DMBP-based point-of-care devices more portable and robust.

2. Experimental section

2.1. Fabrication and design of the DMBP-based chip

As schematically shown in Fig. 1, the DMBP-based PDMS chip consists of three elements: the DMBP, the reaction chamber, and the capillary pump. The DMBP is fabricated in the sample inlet by a method of natural deposition of beads. The reaction chamber is 1.5 mm long, 0.25 mm wide, and 50 μm deep. Compared with the reaction chamber, the capillary pump contains many smaller flow paths (see Supplementary data, Fig. S1 for parameters), which enables to increase capillary pressure. The increased capillary pressure facilitates to the elevation of the plasma extraction rate. Moreover, the capillary pump located at the end of the flow path also acts as a waste collector.

The surface-embossed PDMS chip was fabricated by soft lithography technology [33]. Briefly, a SU-8 mold was firstly fabricated using standard photolithography technology. The PDMS precursor and curing agent (Sylgard 184, Dow Corning, USA) were thoroughly mixed in a weight ratio of 10:1 and degassed in vacuum. The curing mixture was poured onto the SU-8 mold to a final thickness of 2.5 mm and cured in an oven for 2 h at 80 °C. The PDMS slab was peeled off from the mold and cut by a knife to make the opening inlet and outlet (at the terminus of the capillary pump). To produce a capillary-driven flow, the surface-embossed PDMS slab was treated by oxygen plasma for 1.5 min, which renders surface of PDMS hydrophilic and reduces the non-specific absorption. Finally, this PDMS slab was placed orthogonally to the antibody strips that were immobilized onto the plain PDMS substrate in advance.

2.2. Fabrication of the DMBP and incorporation of conjugate reagents

In this study, we incorporated conjugate reagents into the DMBP during the assembly of the DMBP. Silica beads (Sphere scientific corporation, China) with 4 μm in diameter were used to prepare bead slurry in the conjugate reagent solution containing 1% bovine serum albumin (BSA), 5% trehalose, 200 μg mL^{-1} FITC-conjugated goat anti-human IgG (g-hIgG) in PBS. The end concentration of bead slurry was 100% (W/V). Bead slurry was gently agitated prior to each loading. A drop of bead slurry was loaded on the opening sample inlet. Slurry was drawn into the channel by the capillary force and dried in air at the room temperature. With evaporation of water, beads naturally were deposited onto the channel to form a compact DMBP, while conjugate reagents were deposited onto the surface of beads simultaneously. Although the drying process could be influenced by temperature, humidity, and the concentration of the introduced bead slurry, the drying was rapid and usually completed within 2 min. After the formation of the DMBP, residual beads near the sample inlet were removed carefully. The length of the DMBP was ~1.2 mm, which can be controlled by adjusting the volume of introduced bead slurry.

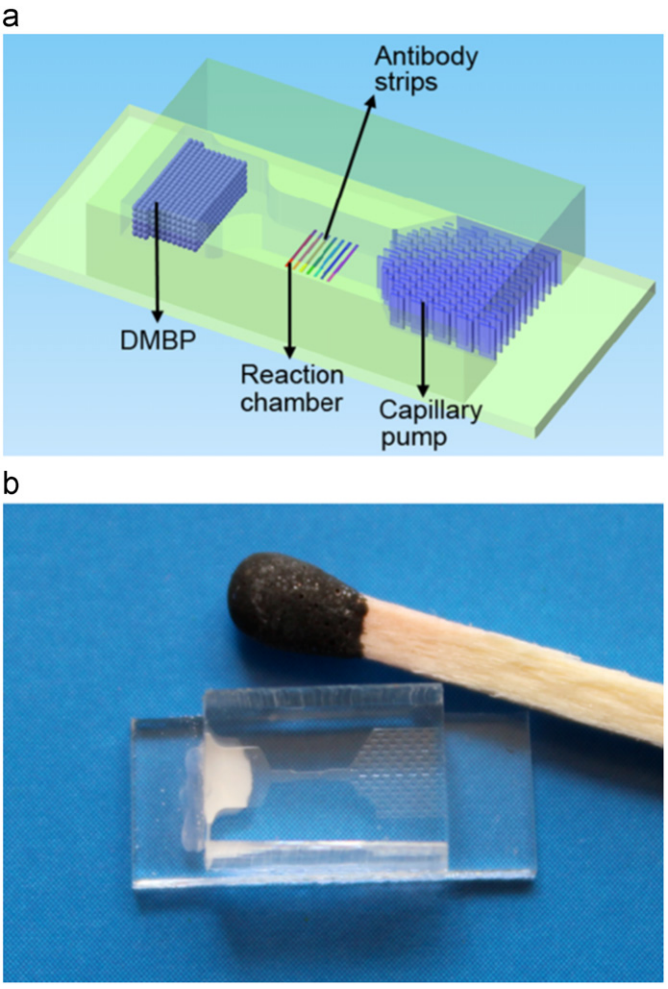

Fig. 1. (a) Schematic diagram of a DMBP-based microfluidic chip. This chip contains three elements: the DMBP, the reaction chamber, and the capillary pump. The DMBP is used as a filter for plasma extraction and a carrier for conjugate reagents; the reaction chamber enables to accommodate multiple antibody strips; the capillary pump provides fluid propulsion using capillary force. (b) Photograph of the chip.

2.3. Immobilization of antibody strips

The patterning of antibody strips involved the following steps: (1) a pre-fabricated PDMS slab containing parallel microfluidic channels was treated with oxygen plasma, and then incubated in 3% BSA solution for 30 min at the room temperature, rinsed with