Talanta 175 (2017) 483–487

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Stacking chip for quantitative bioanalysis

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ARTICLE INFO

Keywords:
Microfluidics
PDMS
Microwells
Enzyme assay
Multistep reaction

ABSTRACT

This paper describes a microwell-based microdevice for performing quantitative bioanalysis. This microdevice combined the passive pumping by degassed polydimethylsiloxane (PDMS) with serial operations including solution dispensing, plates splitting and plates stacking. We name this microdevice "stacking chip". To use the stacking chip in quantitative bioanalysis, nanoliter solutions were first dispensed into the microwells through the degassed PDMS microchannels. Next, we split the microwell and microchannel plates assisted by the application of one drop of silicone oil, which resulted in a microwell array containing the reagent solutions. Microreactor arrays were formed by stacking the two microwell arrays containing the reagent solutions. With this microdevice, the enzymatic kinetics of alkaline phosphatase during the dissociation of the fluorescein diphosphate was measured and analyzed by the Michaelis-Menten model. The stacking chip is simple to fabricate and operate, and amenable to automation for high throughput analysis.

1. Introduction

Microwell plates have become a standard tool in the research laboratory and in clinical diagnosis testing. An important trend of the plate evolution is the further miniaturization of the microwells to achieve automated and high throughput analysis [1,2]. The miniaturized microwell plates with nanoliter (nL) to picoliter (pL) level reagent consumption have been applied in much analytical research [3–25], including digital PCR [5–8], protein synthesis [9], protein crystallization screening [10–12], enzymatic assay [13–17], mammalian cell and stem cell culture [20–25], and so on. As the size of the microwells decreases, the risk of cross-contamination and the difficulty of operation increase substantially.

Previously, our lab developed a simple nanoliter microwell plate based microfluidic system [11]. We combined the passive pumping by degassed polydimethylsiloxane (PDMS) [26–30] with serial operations, including solution dispensing, plates splitting and plates stacking to form the microreactor array. We applied the microfluidic system to screening the conditions of protein crystallization [11,30]. In the present work, we revised the design and operation to make the microfluidic system more robust and with a new range of applications in high throughput analysis. We named this improved microfluidic system "stacking chip" [11]. To illustrate the application, we applied the stacking chip to the steady-state kinetics measurement, which is a routine quantitative bioanalysis essential to enzyme assays. We also demonstrated that the stacking chip could be automated for the high throughput analysis. High throughput kinetics measurement is an increasingly important feature for the development of the functional metagenomics studies [31,32] and protein engineering [33,34], which leads to the increasing number of enzymes.

2. Experimental

2.1. Device design and fabrication

The stacking chip consists of microchannel plate and microwell plate. The microchannel plate was made in PDMS. The microwell plate could be made in PDMS, polymethylmethacrylate (PMMA) or glass. The microchannel and microwell plates were reversibly bound with alignment by naked eyes or with the assistance of an optical microscope.

The PDMS plates were fabricated by soft lithography [35,36]. PMMA microwell plates were fabricated by drilling PMMA slides using a drill press with a cobalt micro-drill bit with the diameter of 350 μm. The drill press was mounted on the home-made three-dimensional motion stage, which was computer controlled.

Two whole-PDMS microchips were used in this work, one for the quantitative analysis (4 × 4 microwell array) and the other for the dispensing and splitting demonstration (3 × 3 microwell array). For the micro-dispensing of multiple reagents, the micro-dispensing system consists of a PDMS microchannel plate, a PMMA microwell plate, a PMMA alignment assistant plate and a PMMA reservoir plate.

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http://dx.doi.org/10.1016/j.talanta.2017.07.077
Received 20 May 2017; Received in revised form 21 July 2017; Accepted 24 July 2017
Available online 25 July 2017
0039-9140/

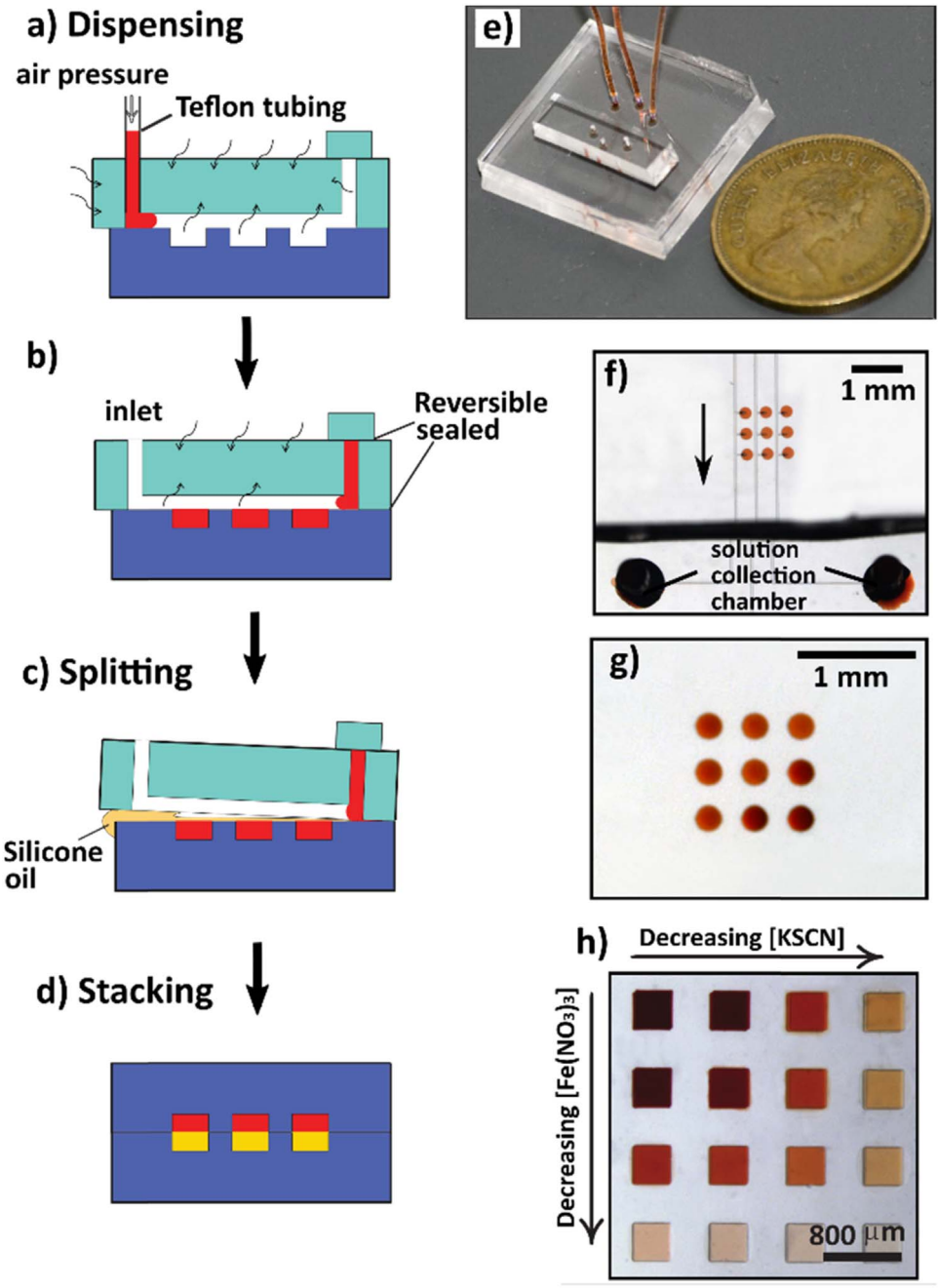

Fig. 1. (a–d) The schematic illustration of the operation of the stacking chip: a,b) the reagents are infused into the microwell array through the reversibly sealed PDMS microchannels by degassed PDMS pumping. c) A drop of silicone oil is added to prevent the liquid evaporation during plates splitting. d) Two microwell plates are stacked to form reaction microreactor array. (e–g) Demonstration of the stacking chip with a 3 × 3 array of microwell plate: e) Photograph of the microchip system next to a Hong Kong fifty-cent coin. f) Microphotograph of the microchannels and the microwells with 0.1 M $Fe(SCN)_x^{3-x}$ solution after dispensing. g) Microphotograph of the microwells filled with $Fe(SCN)_x^{3-x}$ after the removal of the microchannel plate. h) A microphotograph of a 4 × 4 array of the stacking microwell plates containing $Fe(SCN)_x^{3-x}$ solutions with a concentration gradient along both horizontal and vertical directions.

2.2. Reagents preparation and dispensing

Alkaline phosphatase (Sigma-Aldrich) was dissolved in 10 mM diethanolamine buffer (pH 10.1), containing 1 mM $MgCl_2$. Fluorescein diphosphate (Sigma-Aldrich) solutions of various concentrations were prepared in 10 mM diethanolamine buffer.

In the stacking chip, the micro-dispensing of nanoliter reagents into the microwells is achieved using the same passive pumping method that we developed previously [11,15,26,27]. The PDMS microchannel plate was reversibly bound with a microwell plate, forming a microchip. The microchip was degassed in a vacuum chamber for 10 min at 6 kPa. The pre-loaded Teflon tubing was inserted into the inlet of the microchannel to start the dispensing, once the microchip was placed back to the atmosphere. After the completion of the dispensing, one drop of 50 μl silicone oil (Fluid 5, Brookfield) was added at the interface between the two plates (Fig. 1a and b). The PDMS microchannel plate was then removed from the microwell plate (Fig. 1c).

3. Results and discussion

3.1. Micro-dispensing of reagents

In the stacking chip, the micro-dispensing of nanoliter reagents into the microwells was achieved using the same passive pumping method