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A volumetric meter chip for point-of-care quantitative detection of bovine catalase for food safety control

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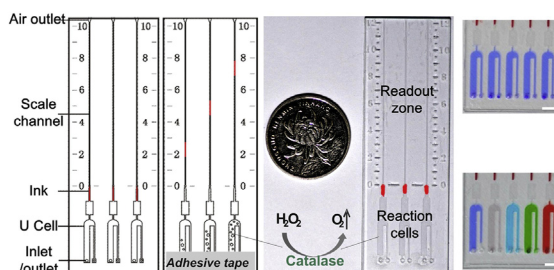
HIGHLIGHTS

- The meter chip is a standalone point-of-care diagnostic tool with visible readouts of quantification results.
- A fast and low cost fabrication protocol (~3 min and ~$0.2 per chip) of meter chip was proposed.
- The chip may hold the potential for rapid scaning of bovine mastitis in cattle farms for food safety control.

GRAPHICAL ABSTRACT



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ABSTRACT

A volumetric meter chip was developed for quantitative point-of-care (POC) analysis of bovine catalase, a bioindicator of bovine mastitis, in milk samples. The meter chip displays multiplexed quantitative results by presenting the distance of ink bar advancement that is detectable by the naked eye. The meter chip comprises a poly(methyl methacrylate) (PMMA) layer, a double-sided adhesive (DSA) layer and a glass slide layer fabricated by the laser-etching method, which is typically simple, rapid (~3 min per chip), and cost effective (~$0.2 per chip). Specially designed "U shape" reaction cells are covered by an adhesive tape that serves as an on-off switch, enabling the simple operation of the assay. As a proof of concept, we employed the developed meter chip for the quantification of bovine catalase in raw milk samples to detect catalase concentrations as low as 20 μg/mL. The meter chip has great potential to detect various target analytes for a wide range of POC applications.

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1. Introduction

In recent years, food safety issues have attracted significant public concerns. Several incidences of food poisoning have been reported, mainly due to the presence of bacterial pathogens in dairy products [1,2]. For example, bovine mammary gland inflammation

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caused by pathogens, namely, bovine mastitis, has significantly affected the dairy industry [3]. The conventional methods used for the detection of mastitis, such as biochemical tests (e.g., catalase test) or serological assays (e.g., enzyme linked immunosorbent assay, ELISA), are expensive and laborious, limiting their use in point-of-care (POC) settings [4,5]. In contrast to laboratory-based assays, microfluidic devices are ideal tools for the detection of foodborne illnesses at the POC due to their portability, cost-effectiveness and ease of use [6–10].

Although significant advancement has been achieved to improve the usability of microfluidic devices, their applications are still hindered by several challenges [11,12]. For instance, conventional assembly and fabrication of microfluidics normally involve lithography technologies, which require clean rooms, meticulous material selection and complex manufacturing that significantly limit large-scale production [13]. Regarding fluidic control and signal detection, the requirement for large equipment, such as a cumbersome pump/valve controller and complex optical and/or electrical analyser, has added to the cost and complexity of the assay. Therefore, there is still a challenge to develop an inexpensive microfluidic system with simple fabrication and operation processes that is compatible with rapid POC assays.

Numerous efforts have been made to address these issues. For example, to simplify the fabrication process, laser etching has been introduced to substitute for the conventional techniques (*e.g.*, elastomeric micromoulding and lithography techniques) that are used for the fabrication of silicon or PDMS microfluidic chips [14–16]. Moreover, utilizing small passive pumps as substitutes of large electronic pumps for fluidic control has greatly improved the portability of devices [17–19]. In addition, cost-effective optical detectors and smartphones have been introduced for signal detection, eradicating the need for high-end optical equipments [20,21]. More recently, several studies have reported the development of miniaturized microfluidic devices (*e.g.*, microfluidic thread-based analytical device (μTAD) [22–24] and a volumetric bar chart chip (V-Chip) [21,25]), which enable target quantification by the naked eye through the visualized distance change of the indicator without the requirement for any external detectors. For example, a μTAD was introduced for the quantification of Ni^{2+} in water by measuring the colour change length on the threads [24]. However, the difficulty in determining the two ends of the coloured lengths caused inaccurate quantification, and a ruler is still required to measure the colour change in the threads. In another study, a V-chip was also demonstrated for multiplexed target quantification by presenting a visualized bar chart on the chip [21], but the requirement for complex fabrication (*i.e.*, photolithography and glass etching) and operation processes (*i.e.*, sliding the glass plates) may limit their applications in POC testings.

In this paper, we developed a microfluidic volumetric meter chip that was fabricated using a laser-etching method. The integration of a specially designed “U shape” reaction cell and an adhesive tape that acts as a simple on-off switch enable the simple operation of the device. Additionally, the multi-channel meter chip with a presented scale allows for rapid multiplexed target quantification by presenting the distance of the ink bar advancement that is detectable by the naked eye, eliminating the need for an extra bulky optical detector. We demonstrated the application of our prototype in food safety analysis using bovine catalase spiked into milk as a target analyte with a limit of detection as low as 20 μg/mL. We envision that the meter chip could be broadly applied to the detection of other target analytes at the point of care, holding great potential for a wide range of applications.

2. Experimental section

2.1. Materials

Bovine catalase (stored at −20 °C), H_2O_2 (30% W/V) and mineral oil were obtained from Sigma-Aldrich (St. Louis, MO, USA). PMMA plates (thickness = 0.5 mm) were purchased from MEIHAN SHINKU KOGYO Co, LTD. (Osaka, Japan). Double-sided adhesive (DSA) tape was purchased from 3 M (#8146–2, thickness = 0.05 mm, Optically Clear Adhesive). Sail brand microscopic slides (76.0 × 25.4 × 1.0 mm) were obtained from a local supplier. PBS tablets (without calcium and magnesium) were bought from MP Biomedicals, LLC. All of the solutions used in this study were prepared with ultrapure water (>18 MΩ cm) from a Milli-Q Integral Water Purification System (Merck Millipore Co., Darmstadt, Germany).

2.2. Chip design and fabrication

The microfluidic meter chip was designed with CorelDRAW X3 software and was manufactured using a desktop laser-cutting machine (VersaLASER®; Lens 2.0, Universal VLS 2.30) with a resolution at 70 μm. Each device consisted of three layers—a PMMA plate, an adhesive layer and a glass slide (Fig. 1a). In the working unit, reaction cells provided a space for biomolecule reactions that finally generate gas. To avoid possible failure of the assay due to the rapid mixing of reagents and red ink during the reaction process, small chambers were created (Fig. 1a) to separate the reagent in the reaction chamber (*e.g.*, hydrogen peroxide (H_2O_2)) from the ink deposited in the microchannels before the assay begins.

Using the laser-etching method, the first layer, the PMMA plate, was etched by a laser into pieces that were 75 × 25 × 1 mm with engraved inlets and outlets and meter scales (Fig. 1a). To prepare the middle layer (*i.e.*, DSA layer), one side of the protective plastic of the DSA was removed manually and then the exposed adhesive film directly etched by the laser beam to create the desired channel pattern. The third layer (*i.e.*, glass slide with the same size of the engraved PMMA plate) was immersed into a piranha solution (H_2SO_4: H_2O_2 = 7:3, V/V) for 1 h, rinsed and dried at room temperature.

Regarding the chip assembly, the DSA and glass slide were first assembled, followed by the insertion of ~0.1 μL of the ink indicator by pipetting into each channel, as illustrated in Fig. 1c. The channel was then covered by PMMA to produce a complete chip.

2.3. Evaluation of the meter chip

Bovine catalase in PBS buffer (concentration various from 0.5, 16, 32, 50, 58, 64 and 128 μg/mL) and a H_2O_2 solution (0.15, 0.45, 0.75, 1.05, 1.35 and 1.65 M) were prepared for testing. Briefly, approximately 0.5 μL of bovine catalase in PBS buffer and 0.5 μL of H_2O_2 was first mixed in a tube and then immediately introduced into the chip through an inlet. When all of the U reaction cells were loaded, a piece of adhesive tape was used to immediately seal all of the inlets/outlets to create a closed environment for the biochemical reaction. To demonstrate the stability of the assay readout after peeling off the tape, bovine catalase enzymatic reactions in PBS (128 μg/mL) and H_2O_2 (0.45 M) were used to perform the test. The concentrations of bovine catalase and H_2O_2 were also optimized based on the advancement of the ink bar.

2.4. Bovine catalase spiked in milk samples

To demonstrate the potential application of our prototype to the detection of bovine catalase in milk for food safety control, bovine

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