Paper-based isothermal DNA amplification and real-time analysis

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***Abstract*— This work reports a paper-based sensor technology for point-of-care nucleic acid amplification tests. The technology integrates a sensor chip and an Arduino-compatible fluorescence detector. The paper-based sensor provides the functions of reagent storage, sample transportation, and DNA amplification. The compact and low-cost fluorescence detector can quantify the amplicons in real time. The results show that the paper-based sensor can detect genomic DNA extracted from *Escherichia coli* with the concentration as low as 2×10^4 copies/ µL. As a diagnosis tool, the sensor has the potential to measure multiple target genes simultaneously.**

Keywords—paper-based sensor; isothermal PCR; nucleic acid test; fluorescence detection

I. INTRODUCTION

As a potential replacement of lab-based testing, point-of-care testing (POCT) allows users to obtain testing results in real time and near the location of patients [1, 2]. On the other hand, nucleic acid amplification tests (NAATs), which are based on the amplification and detection of DNA or RNA sequences associated with target diseases, are one of the most widely used molecular diagnostic tests. For example, the existing NAATs have demonstrated high specificity and sensitivity for the diagnosis of infectious diseases [3, 4]. Real-time polymerase chain reactions (PCRs) and isothermal PCRs, such as the loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification, are the common NAATs for diagnosis. Recently, paper-based NAAT sensors have been investigated for POCT applications [5-7]. A quantitative NAAT requires the real-time detection of the PCR products to generate the amplification curves. By and large, the period of time for the DNA amplification varies linearly with regard to the initial DNA template concentration.

To date, the real-time NAAT analysis on paper is still an unfulfilled task. To address this challenge, we developed an automated and low-cost system using an Arduino microcontroller to facilitate paper-based LAMP detections. Moreover, the integration of two paper materials allows the storage of LAMP reagent, transportation of samples, amplification of target genes, and detection of amplicons in the same paper sensor chip. In conjunction with the compact fluorescence detector, the paper-based sensor chip has the potential to become a POCT diagnosis tool.

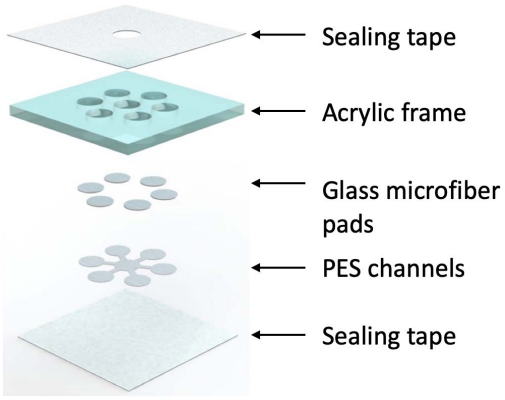

Fig. 1. Schematic illustration of the paper-based LAMP sensor chip.

II. MATERIAL AND METHODS

A. Paper microfluidics-based LAMP sensor

The paper-based LAMP sensor consists of two types of paper substrates and a plastic case as illustrated in **Fig. 1**. The glass microfiber paper (28297-984, VWR International) was used to store the LAMP reagent and host the isothermal amplification reactions. The purchased glass microfiber paper substrate was punched to form 3-mm-diameter pads, in which the LAMP reagent was dried and stored. The polyethersulfone (PES) membrane (PES509025, Sterlitech Corp.) was chosen to connect the inlet to multiple glass microfiber pads. The PES microfluidic layer was patterned into the desired channels using a cutting tool (CM350, Brother). To prevent sample evaporation during LAMP experiments, the paper layers were sealed inside the plastic case. The plastic case was assembled using a perforated acrylic sheet and two layers of plastic sealing tapes. The acrylic sheet was 1.5 mm thick and punched with several 3-mm-diameter holes. The glass microfiber pads and patterned PES membrane were placed inside the holes and below the acrylic sheet, respectively. The top and bottom sides of the

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sensor were covered using a transparent tape (AB0558, Thermo Fisher Scientific).

B. *Compact fluorescence detection system and data processing scheme*

Fig. 2 shows the design of the compact detector. The detector implements two main functions, including the fluorescence imaging and temperature control. The detector was controlled using an Arduino microcontroller, which communicates with a PC via WiFi network. The results of LAMP experiment were quantified using the fluorescence intensity of SYBR green dye, whose emission intensity is proportional to the concentration of the double-stranded DNA (dsDNA) modules [8]. The fluorescence imaging unit consists of an array of blue LEDs (P_{out} = 360 mW) and an Arduino compatible camera (OV5642, ArduCam). The excitation and emission filters (FGB25 and FGL530, Thorlabs Inc.) were jointed and placed in front of the assembly of the camera and LED. The temperature control unit consists of a thermoelectric controller (TEC) and a thermocouple (K-type, Omega Engineering). The TEC was powered using a DC power supply, which was switched by the Arduino microcontroller. During LAMP experiments, the temperature was maintained at 65 °C and fluorescence images were measured every 120 sec.

Acquired fluorescence images were transferred to a computer. The program has been developed to extract the green channel of RGB images, identify the LAMP reaction pads using the K-means clustering algorithm, and calculate the average fluorescence intensity in the pad regions [9]. For each sample pad, the average fluorescence intensities were plotted as a function of time to generate the amplification curves.

C. *LAMP reagent and preparation of genome DNA*

The LAMP primers were designed to detect the *malB* gene of *Escherichia coli* (*E. coli*) [10]. The LAMP primers: out primer F3 (GCCATCTCCTGATGACGC, 0.2 µM), out primer B3 (ATTTACCGCAGCCAGACG, 0.2 µM), loop primer F loop (CTTTGTAACAACCTGTCATCGACA, 0.4 µM), loop primer B loop (ATCAATCTCGATATCCATGAAGGAG, 0.4 µM), inner primer BIP (CTGGGGCGAGGTCGTGGTATTC CGACAAACACCACGAATT, 1.6 µM), and inner primer FIP (CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAAT GTTGCT, 1.6 µM). The LAMP master reaction mix, containing the *Bst* 3.0 DNA polymerase, dNTP, SYBR green, and isothermal amplification buffer, were purchased from New England Biolabs, Inc. The LAMP reagent (10 µL) was injected into the glass microfiber pads and dried using a vacuum oven. The genomic DNA was extracted from *E. Coli* through cell lysis, precipitation, and purification using a DNA purification kit (A1120, Promega). The concentrations of purifed DNAs were quantified by the NanoDrop (Thermo Fisher Scientific).

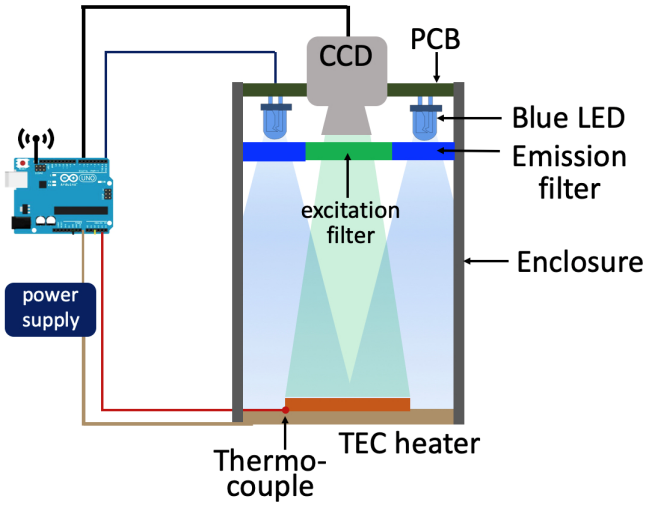

Fig. 2. Schematic diagram of the compact and real-time LAMP detection setup.

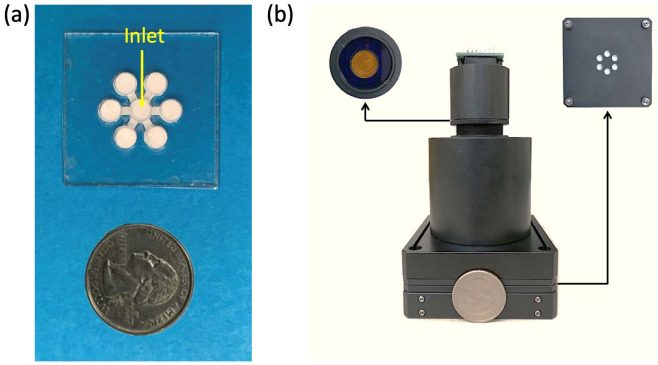

Fig. 3. Photographs of the fabricated paper-based LAMP sensor and the compact detector.

III. Results and Discussion

The fabricated paper LAMP sensor and the compact detector are shown in **Fig. 3(a)** and **(b)**, respectively. The paper sensor chip includes six reaction chambers that have the capacity of amplifying six different genes simultaneously. For a multiplex LAMP detection, the primer pairs for a gene needs to be stored in the specific glass microfiber pad. The compact detector was built using the lens tubes as the enclosure to block unwanted ambient light. The fluorescence imaging components, including the Arduino camera, LEDs, and filters, reside in the top compartment. The paper sensor chip and temperature controller located on the bottom of the base. The left inset of **Fig. 3(b)** shows the photo of the joined emission and excitation filters in front of the camera. The right inset of **Fig. 3(b)** is the photo of the paper LAMP sensor taken through a black shadow mask. The mask was installed to minimize the reflection of the LED excitation by the plastic case.

Extracted genome DNAs were pipetted into the inlet hole (**Fig. 3(a)**) and transported to the glass microfiber pads through the PES flow channels. We used green ink to illustrate the sample flow process along through the papers. As shown in **Fig. 4(a)**, the green ink wicked into the reaction chambers within 1.5 minutes. During the sample transport on the paper sensor, the

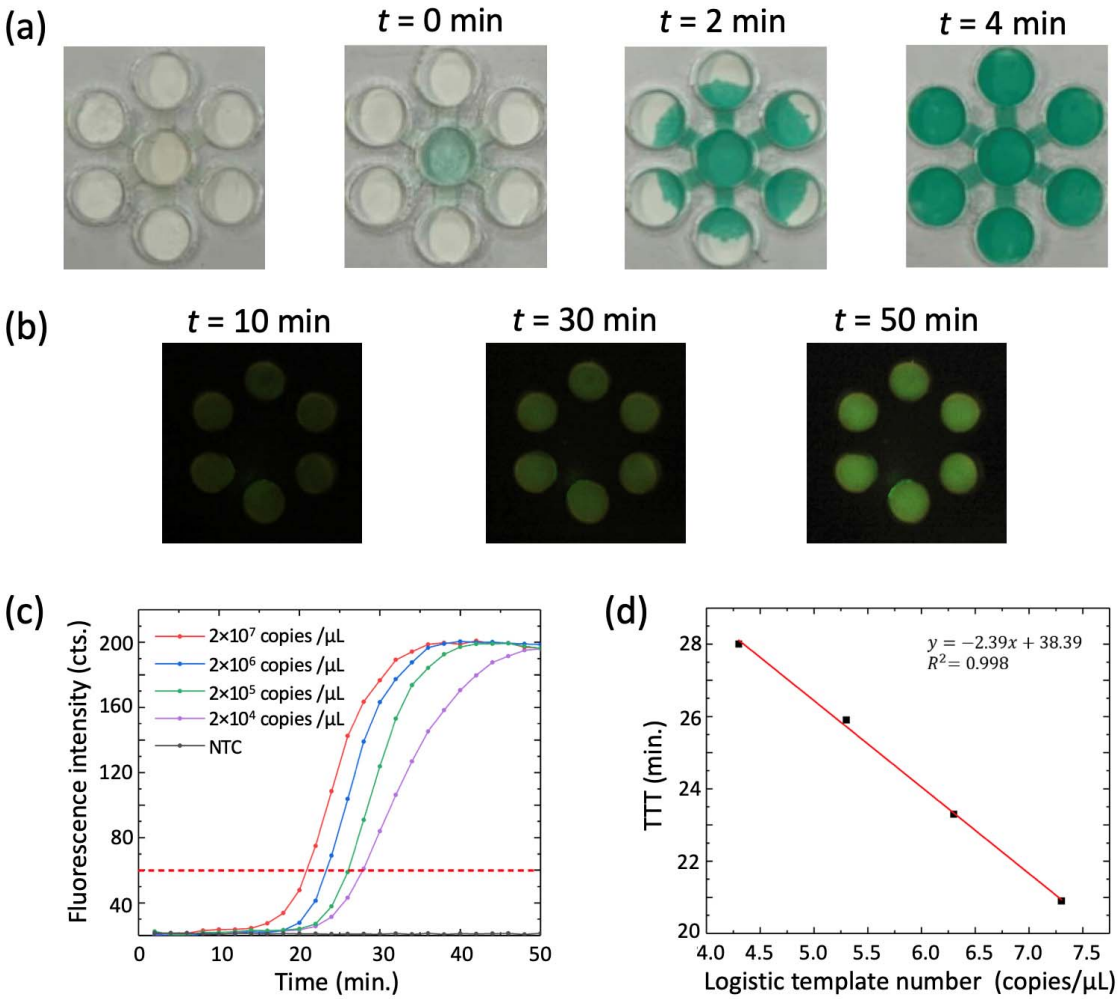

Fig. 4. Results of the paper-based LAMP test in real time. (a) Sample transportation in the paper-based sensor. (b) Fluorescence images captured during the isothermal amplification. (c) Real-time amplification curves for different concretions of template DNA extracted from E. coli. (d) Calculated TTT values for different DNA concentrations.

glass microfiber served as the absorption pad to facilitate the sample flow.

When the glass microfiber pads, in which the LAMP reagents were stored, were fully soaked by the *E. coli* DNA sample, the temperature controller started to raise the temperature to the desired temperature of the LAMP reaction. Here, the reaction temperature was maintained at 65 °C. **Fig. 4(b)** shows the fluorescence images of the sample with 2×10^5 copies/μL genomic DNA, taken at 10 min, 30 min, and 50 min respectively. The increase of the SYBR green emission indicates the successful production of double-stranded DNA amplicons.

The captured fluorescence images were analyzed using the K-means clustering algorithm to identify the LAMP reaction chambers. The fluorescence intensity of each chamber was calculated and plotted as a function of reaction time. **Fig. 4(c)** summarizes the LAMP amplification curve of the *malB* gene at four different concentrations ranging from 2×10^4 to 2×10^7 copies/μL. In **Fig. 4(c)**, the red dashed line represents the pre-set intensity threshold of 60 cts.. The time to threshold (TTT) value is the time required for the LAMP output to exceed the threshold value [11]. The amplification curves show that the TTT values increase as the decrease of the DNA template concentration. **Fig. 4(d)** plots the TTT values for the DNA concentrations. The result suggests that the paper-based real-time LAMP sensor chip has the potential to become a quantitative analytical tool.

We are currently investigating and optimizing the process to dry the LAMP reagent and primers in the glass microfiber pads. The stored LAMP reagents in the reaction pad will functionalize glass microfiber pads and allow multiplex detection of different targets within our compact setup. The results will be reported at the conference.

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