Molecular and Cellular Probes 29 (2015) 223–227

Contents lists available at ScienceDirect

Molecular and Cellular Probes

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

An integrated microfluidic chip for the detection of bacteria – A proof of concept

Zhe Guo [a, *, 1], Ting Yu [a, 1], Jiarui He [b], Fen Liu [a], Hualong Hao [a], Yang Zhao [a], Jiabin Wen [a, *], Qi Wang [a, *]

[a] Department of Respiratory Medicine, The Second Hospital, Dalian Medical University, Dalian 116023, China
[b] Beijing Institute of Respiratory Medicine, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100069, China

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form
9 May 2015
Accepted 11 May 2015
Available online 13 May 2015

Keywords:
Microfluidic chip
Solid phase extraction (SPE)
Loop-mediated isothermal amplification (LAMP)
Bacterial detection

ABSTRACT

We designed a microfluidic chip as a proof of concept for the detection of bacterial DNA. The chip was fabricated with poly-dimethylsiloxane (PDMS). It included a solid phase extraction (SPE) chamber, two separate channels and multiple loop-mediated isothermal amplification (LAMP) chambers. Three bacterial strains (*Escherichia coli O157*:*H7*, *methicillin-resistant Staphylococcus aureus* and *methicillin-sensitive S. aureus*) were used to test the feasibility of the device. LAMP products were examined directly using a UV light and verified by agarose gel electrophoresis. Using this chip, we successfully detected *E. coli O157*:*H7*, *MSSA* and *MRSA* in less than 2 h. The detection limit for genes *rfb*E, *spa* and *mec*A (specific to *E. coli O157*:*H7*, *MSSA* and *MRSA*, respectively) was $<10^2$ CFU/100 μl. Further work is required to refine this approach and rigorously assess its analytical and diagnostic specificity and sensitivity.

1. Introduction

Bacterial disease is one of the most important health issues worldwide, and can result in high morbidity and mortality as well as high costs for treatment [1]. The leading causes of infection include gram-negative and gram-positive bacteria. *Escherichia coli O157*: *H7*, a common gram-negative bacterium, is recognized as one of the most important food-borne pathogens [2,3]. *Staphylococcus aureus* is one of the most important gram-positive pathogens associated with community-acquired and hospital-acquired bloodstream infections [1]. Moreover, the mortality of patients infected with *methicillin-resistance S. aureus* (*MRSA*), a strain resistant to penicillin and β-lactam antimicrobial drugs [4], is twice that than for patients infected by *methicillin-sensitive S. aureus* (*MSSA*) [5,6].

Traditional diagnosis of bacterial infections, including antimicrobial susceptibility testing and cell culture, can take more than 72 h, during which patients are treated with broad range therapies, resulting in higher health care costs and increased antibiotic resistance [7]. Therefore, it is important to develop accurate and rapid methods for bacterial detection. Some polymerase chain reaction (PCR) methods have been shown to accurately diagnose bacteria with high sensitivity and specificity [8–10]. However, PCR requires expensive thermal cyclers and can be time-consuming to perform. To overcome these limitations, a novel nucleic acid amplification method, called loop-mediated isothermal amplification (LAMP), was developed by Notomi et al. [11]. LAMP relies on an auto-cycling strand displacement DNA synthesis using the *Bst* DNA polymerase large fragment. It involves a set of 4–6 primers, which recognize 6 to 8 distinct regions on the target gene. It takes only 30–60 min at a constant temperature (60 °C–65 °C). Furthermore, LAMP reaction generates a large amount of by-product, such as pyrophosphate ion [12], which can be detected using the fluorescence dye calcein [13]. Therefore, LAMP assay has been regarded as a useful detection tool and can achieve acceptable efficiency, specificity and sensitivity.

The efficiency of DNA extraction has also been improved by the recent development of a silica-based solid phase extraction (SPE) method [14–16], which relies on the interaction between DNA and the silica surfaces in the presence of a high chaotropic agent, such as guanidine thiocyanate (GuSCN).

To further simplify diagnosis, microfluidic chips have been investigated in recent years [17,18]. The microfluidic systems might

* Corresponding authors.
E-mail addresses: wenjiabin2005@sina.com (J. Wen), wqdlmu@163.com (Q. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mcp.2015.05.005
0890-8508/

be more cost effective and achieve higher levels of integration [19–22]. However, improvements in the microfluidic systems are still required. Here, we designed and assessed a double-layer microfluidic chip which can be used for the detection of bacteria. Using this device, we isolated bacterial DNAs by binding to silica beads, amplified target genes by LAMP and directly detected products on the same chip by calcein staining.

2. Materials and methods

2.1. The microfluidic chip

The schematic representation of the double-layer device is shown in Fig. 1A. The microchip was fabricated with polydimethylsiloxane (PDMS) (Dow Corning, USA) by standard soft lithography methods [23,24]. The microchip was fabricated by replica molding PDMS against the masters, and the upper and lower layers of the microchip and a glass slide were irreversibly bonded together in sequence via oxygen plasma surface treatment (150 mTorr, 50 W, 20 s). The upper layer of the double-layer chip was used to isolate DNA from the samples. It consisted of seven inlets, one outlet and one SPE chamber. Inlet A was used to load the reagents of DNA extraction, samples and silica beads into the SPE chamber. The gaps on the micro-pillars were 60 μm wide, smaller than the silica beads (average diameter = 70 μm) (Guangyao New Material Co., China), so that the silica beads were blocked from crossing the SPE chamber (Fig. 1C). As a result, only the solution pumped from inlet A could move to either outlet, or the LAMP chambers. On micro-channels A and B, two small clips were employed as micro-valves to control the loading of solution into the LAMP chambers (Fig. 1D) [25]. The lower layer of the chip was composed of six LAMP reaction chambers, and chambers 1–3 were connected to the SPE chamber and used to for the detection of gene sequences. Chambers 4–6 were isolated and used for control samples. The LAMP reaction mixture, excluding DNA samples and the *Bst* DNA polymerase large fragment, was added from the inlets of the upper layer to the LAMP chambers (chambers 1, 2 and 3). The two layers were combined through matched holes inside the channels of the upper and lower layers. The microfluidic chip was treated with distilled water and then autoclaved at 120 °C and 103.4 kPa for 30 min before use.

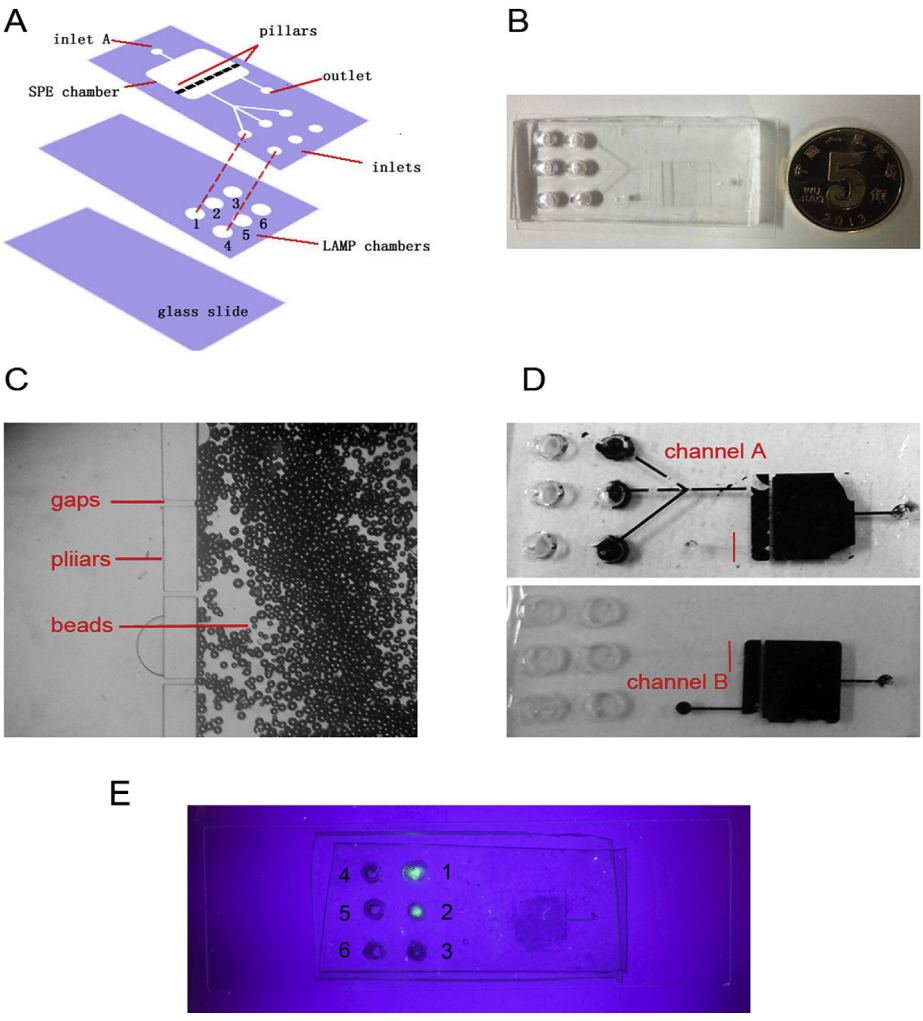

Fig. 1. Microfluidic device design. (A) A schematic chart of the double-layered chip, with a layout of each layer. (B) A photograph of the double-layered microfluidic device. (C) A photograph of the SPE chamber, showing silica beads blocked by the row of micro-pillars and the micro-gaps. (D) A photograph of the solution flow in the chip. Injection of black ink from inlet A flowed to channel A or B, respectively, depending on the opening/close of the clips, which are indicated by the red lines. (E) A representative result of LAMP reaction. Under the UV light, green fluorescence was observed in positive results (chamber 1 and chamber 2), whereas no fluorescence was observed in negative results (chamber 3) or control groups (chambers 4, 5, 6) (purple color indicates the direct emission of the UV light). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)