A magnetic bead-based assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* by using a microfluidic system with integrated loop-mediated isothermal amplification

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Supplimentary information

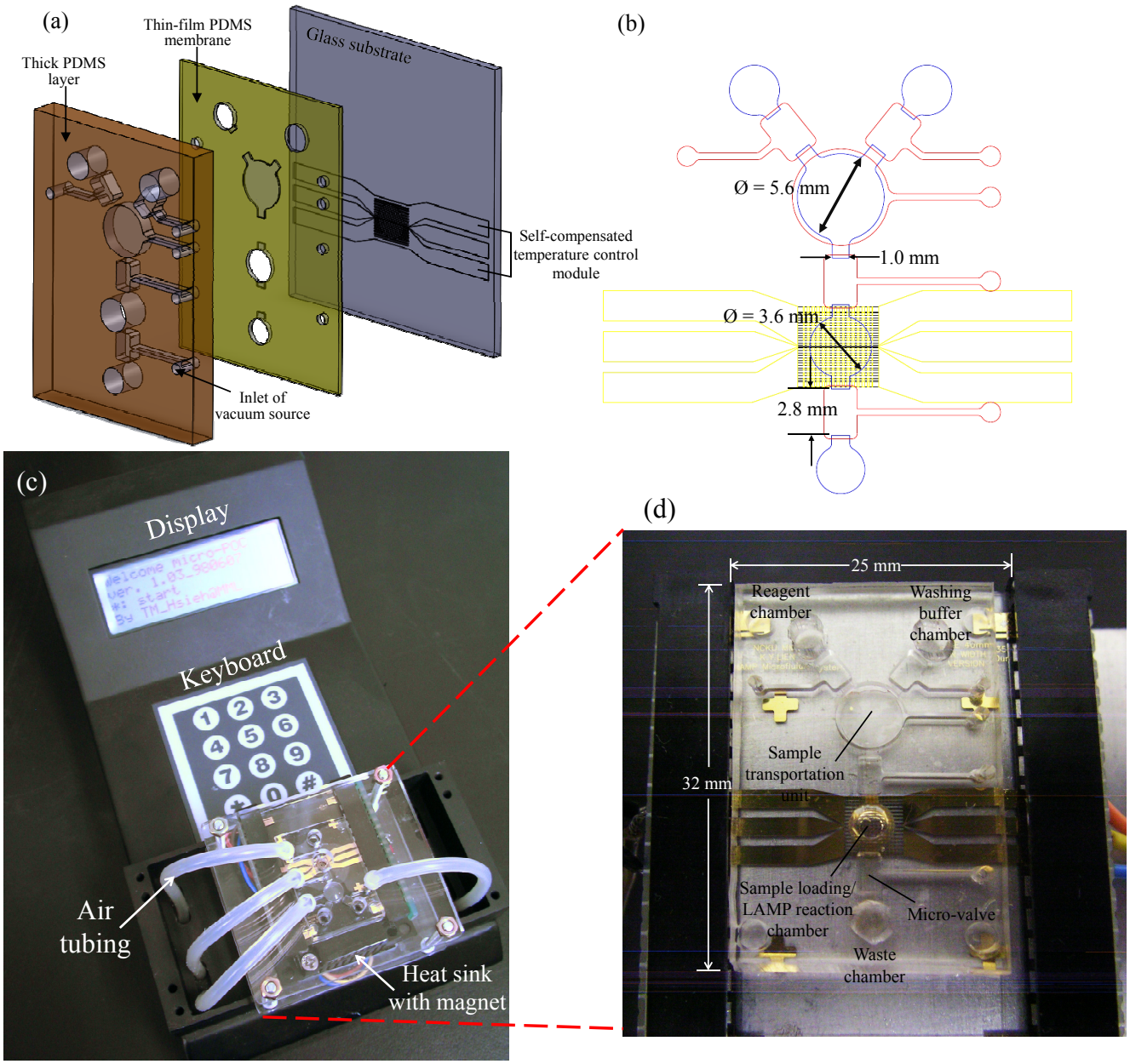

Fig. 1 (a) An exploded view of the microfluidic system consisting of two PDMS layers, including a thick PDMS structure with air chambers and a thin-film PDMS membrane as a fluidic channel layer, and one glass substrate patterned with metal electrodes as micro-heaters and a temperature sensor. (b) Schematic illustration of the magnetic-bead-based, suction-type microfluidic system for rapid detection of MRSA. (c) A photograph of a hand-held, portable system with integration of a control circuit board, EMVs, a vaccum pump and a waste tank. (d) A photograph of the integrated microfluidic system consisting of a sample transportation unit, a waste chamber, a reagent chamber, a washing buffer chamber, micro-valves and a reaction chamber.

Design of the microfluidic system

The entire diagnostic assay for MRSA detection can be performed automatically in a suction-type microfluidic system within 60 minutes. An illustration of the microfluidic device consisting of two PDMS layers, including a thick PDMS structure with air chambers and a thin-film PDMS membrane as a fluidic channel layer, and one glass substrate as the bottom layer is shown in Figure 1(a). A photograph of the integrated microfluidic system consisting of a sample transportation unit, a waste chamber, a reagent chamber, a washing buffer chamber, micro-valves and a reaction chamber is shown in Figure 1(d). The dimensions of the microfluidic compoents are detailed in Figure 1(b). The overall dimensions of the microfluidic chips are measured to be 32 mm ×30 mm ×10 mm. Notably, all external control components such as a control circuit board, electromagnetic valves (EMVs), a vaccum pump and a waste tank have been integrated into a hand-held portable system (as shown in Figure 1(c)). As a consequence, the proposed microfluidic diagnostic assay, incorporated with specific probe-conjugated magnetic bead and on-chip LAMP process, provides a platform to complete the entire diagnosis for an infectious disease in a short period of time with less human intervention.

Fabrication

The microfluidic control module is fabricated by using a computer-numerical-control (CNC) machining process and PDMS casting/replication techniques for fast prototyping of the microfluidic components. Detailed information about the micro-fabrication process can be referenced in our previous work [38]. Briefly, the master molds of PMMA plates with micro-structures are first patterned by using a CNC machine (EGX-400, Roland Inc., Japan) with a 0.5 mm drill bit. The feed speed and rotational rate of the drill bit are 7 mm/s and 26,000 rpm, respectively. This is then followed by a PDMS casting process to form the inverse images of the air chamber mold and the fluidic channel mold. PDMS is prepared by thoroughly mixing the PDMS prepolymer and the curing agent (Sylgard 184A/B, Sil-More Industrial Ltd., USA) in a ratio of 10:1 by weight. The polymer is then de-aerated under vacuum to remove all air bubbles generated during mixing and cured at 100°C for 4 hours. Finally, the two PDMS structures are bonded together by utilizing an oxygen plasma treatment.

In addition to the microfluidic control module, a self-compensated, array-type temperature control module is also integrated to generate a uniform temperature distribution within the reaction chamber. The array-type micro heaters are fabricated using a standard lithography and lift-off process. First, the bio-compatible soda-lime glass is cleaned, followed by patterning a photoresist AZ-4620 layer for the micro heaters and the micro temperature sensor. A standard lift-off process is performed by evaporating 80-nm thick platinum (Pt) as a resistor, which acts asa sensor, utilizing an electron-beam evaporation process. After that, gold (Au) is deposited as the electrical leads of the micro temperature sensor and the micro-heaters for localized heating. Finally, the integrated microfluidic chip is then assembled by bonding the PDMS and the glass substrates together using an oxygen plasma treatment.

Preparation of the standardized staphylococcal genomic DNA

Twenty-seven strains of various types of bacteria including 10 strains of MRSA and 10 strains of MSSA (as listed in Table 1) have been employed for verification of the proposed diagnostic assay. Among them, a standardized staphylococcal genomic DNA of the MRSA strain 1601 is extracted as the DNA templates for investigating optimal conditions for hybridization and the LAMP processes. First, a 100 µL of a bacterial mixture of cultured MRSA is prepared by uiltizing a loop stick to transfer a MRSA colony from a culture plate. This is followed by mixing them with a PBS solution to form the bacterial mixture. It is then boiled at a temperature of 95°C for 10 minutes. The mixture is centrifuged at 12,000 g for 2 minutes, followed by collecting the supernatant solution and re-suspending the DNA templates into a volume of 100 µL with an initial concentration of 10 ng/µL. All the staphylococcal genomic DNA templated is stored at -20 °C prior to usage.

Table 1 Twenty-seven strains of bacterial samples are verified using the microfluidic diagnostic assay

Strains of bacteria	No.	Strains
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	10	1201, 1202, 1203, 1204, 1205, 1206, 301, 1302, 1303, 25923
Methicillin-resistant *Staphylococcus aureus* (MRSA)	10	1519, 1568, 1569, 1570, 1571, 1572, 1573, 1601, 1602, 1603
Streptococcus pneumoniae	1	1645
Escherichia coli	1	57
Enterobacter sp.	1	1124
Klebsiella pneumoniae	1	112
Pseudomonas aeruginosa	1	138

Proteus vulgaris	1	380
Haemophilus influenzae	1	139

Table 2 Designed sequences of the primer sets for the LAMP process and oligonucleotide sequences of the probe for DNA hybridization

Gene	Primer set	Sequences
spa	B3	5'-GCTGATAACAATTTCAACAAAGAAC-3'
	F3	5'-TCATAGAAAGCATTTTGTTGTTC-3'
	BIP	5'-TTGGCTTGGGTCATCTTTTAAGCTCTATGAAATCTTGAATATGCCT-3'
	FIP	5'-TGCTAACCTATTGTCAGAAGCTAAATTTGTTGAATTTGTTATCCGC-3'
	Specific probe	5'-TTTTTTTTTTCGTTATTAGCTGGACGTCGTCGCGAACTATAA-3'
*mec*A	B3	5'-GCAACAAGTCGTAAATAAAACACAT-3'
	F3	5'-TCTCATATAGCTCATCATACACTT-3'
	BIP	5'-CACCCAATTTGTCTGCCAGTTTTATGCAAACTTAATTGGCAAATCC-3'
	FIP	5'-ATCCAAACATGATGATGGCTATTAACCTGAGATTTTGGCATTGTAGC-3'

Table 3 Raw data for the absorbance of OD_{260} from MRSA LAMP amplicons

Conc. of tested DNA	△ absorbance values of OD_{260}			Mean	SD
	I	II	III		
NC	0	0	0	0	0
100 fg	2.02	2.74	3.40	2.72	0.58
1 pg	3.49	3.92	4.64	4.02	0.69
10 pg	7.48	6.13	5.67	6.43	0.94
100 pg	7.54	9.77	8.38	8.56	1.13
1 ng	12.34	10.9	9.89	11.04	1.23