AN INTEGRATED MICROFLUIDIC SYSTEM FOR FAST ISOLATION OF BACTERIA IN HUMAN WHOLE BLOOD FOR DIAGNOSIS OF SEPSIS

Yen-Ling Fang[1], Wen-Bin Lee[1], Chih-Hung Wang[1], Chun-Chih Chien[2], Huey-Ling You[2], Mel S. Lee[3] and Gwo-Bin Lee[1,4,5]***

[1]*Department of Power Mechanical Engineering, National Tsing Hua University, Taiwan*

[2]*Laboratory Medicine, Kaohsiung Chang Gung Memorial Hospital, Taiwan*

[3]*Department of Orthopaedic Surgery, Kaohsiung Chang Gung Memorial Hospital, Taiwan*

[4]*Institute of Biomedical Engineering, National Tsing Hua University, Taiwan*

[5]*Institute of NanoEngineering and Microsystems, National Tsing Hua University, Taiwan*

*e-mail: gwobin@pme.nthu.edu.tw; **email: bone@doctor.com

Abstract

Rapid diagnosis of sepsis assists clinicians to initiate timely antibiotic therapy and therefore reduces mortality of bacterial infections. In this work, we presented an integrated microfluidic system for fast isolation of bacteria for both Gram-positive and Gram-negative bacteria directly from human whole blood, which may be crucial in the detection of sepsis. First, a deterministic lateral displacement (DLD) device was used for sample pretreatment of whole blood, which performed continuous separation of leukocytes, erythrocytes and bacteria. With this approach, most of the blood cells were removed while bacteria and platelets were collected. Then the isolated bacteria were captured with magnetic beads surface-coated with flexible neck regions of mannose-binding lectin (FcMBL) using a new 3D micromixer. The entire process including sample pretreatment and bacteria isolation could be completed on an integrated microfluidic system within 2 hours, which is significantly less than the one for the traditional method (i.e. 3 days). This is the first time that an integrated microfluidic system capable of detecting bacteria by using FcMBL was reported, which may provide a promising way to diagnose sepsis.

Experimental procedure

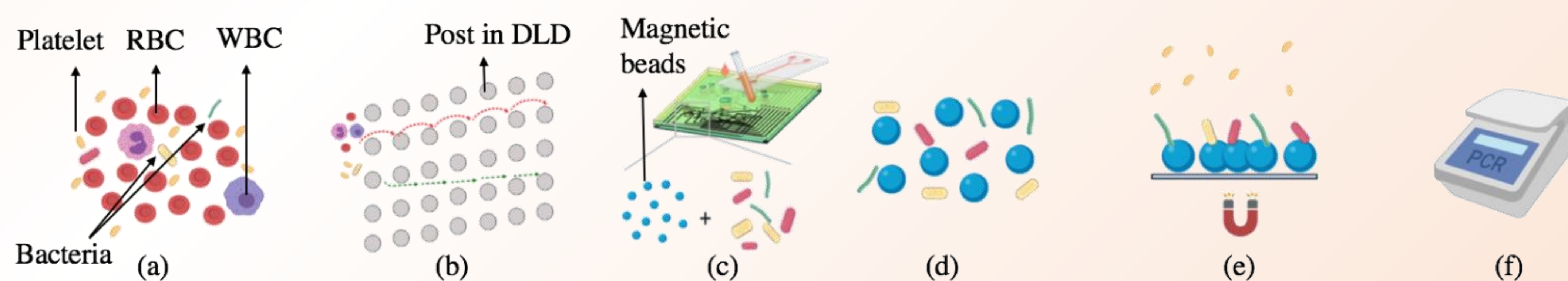


Figure 1: The entire procedure for isolation of bacteria directly from human whole blood. (a) Human whole blood with bacteria. (b) Inject the blood into the DLD chip. Blood cells were deflected and thereby bacteria were isolated continuously. (c) The isolated bacteria were moved to a 3D micromixer underneath. (d) After gentle mixing, bacteria were captured by magnetic beads surface-coated with FcMBL. (e) Collect the beads with a magnet. (f) Identification.

Microfluidic chip design

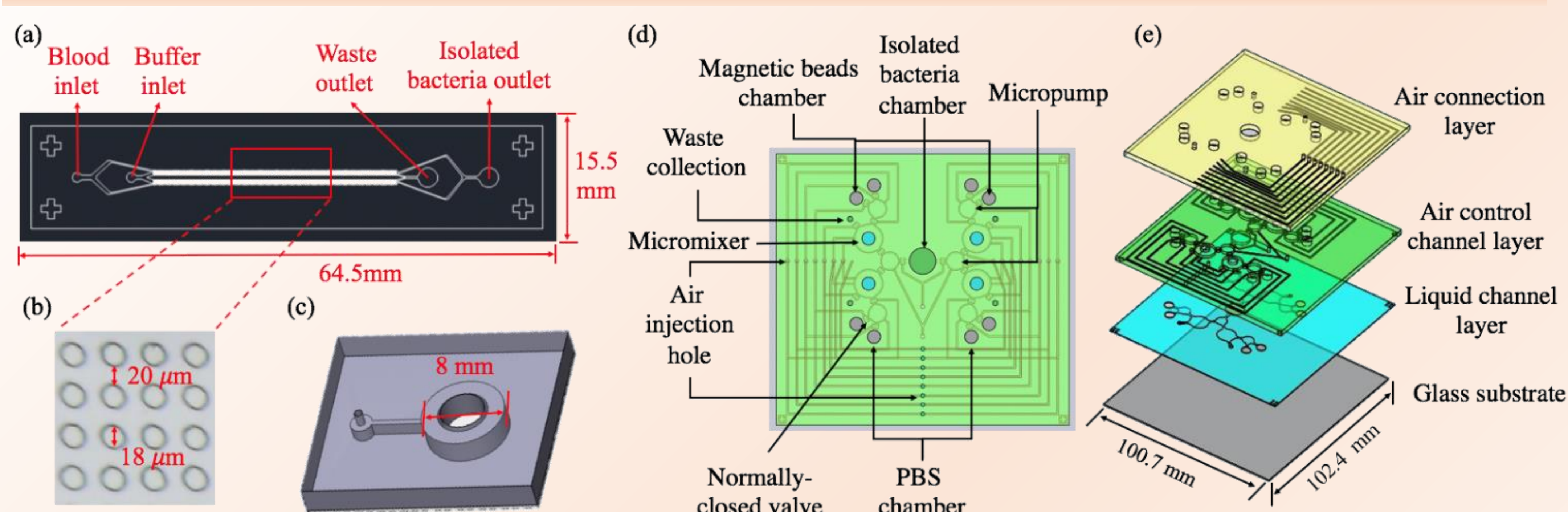
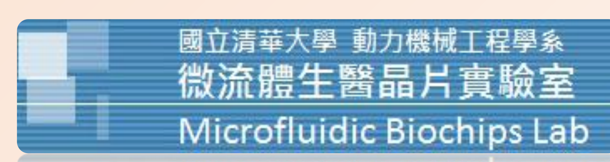


Figure 2: (a) A schematic illustration of the DLD structure. Two inlets were used to inject blood and buffer separately. (b) The pillar structure of DLD under a microscope. Theoretical critical diameter was designed to be 3.7 μm. (c) A schematic illustration of a pneumatically-driven micromixer with a diameter of 8 mm. (d) A detailed design of the integrated microfluidic chip. (e) The chip was consisted of three PDMS layers and a glass substrate.

Acknowledgements

The authors would like to thank Ministry of Science and Technology (MOST) of Taiwan (MOST grants 107-2221-E-007-013-MY3 and Chang Gung Memorial Hospital (Kaohsiung, Taiwan; grants CMRPG3C0231 and CMRPG8E1631) for financial support.



Results

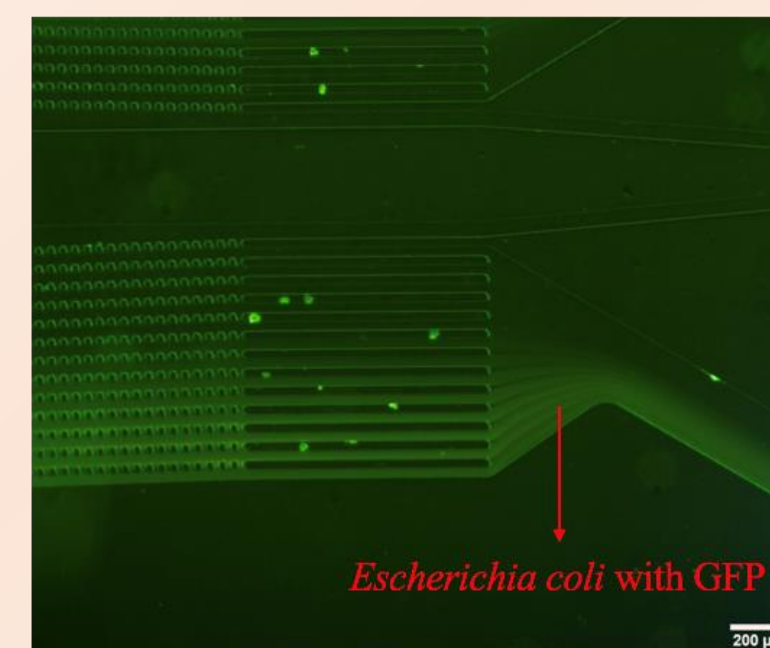


Figure 3: The flow of bacteria around the outlet in the DLD channel. This result showed that all bacteria were not deflected and could be collected from the outlet chamber.

Table 1: The capturing results of FcMBL-coated magnetic beads for 4 kinds of sepsis bacteria.

Supernatant Wash

Captured

	Escherichia coli	*Pseudomonas aeruginosa*
Capture rate in 10^2 CFU/mL	71 %	85 %
	Streptococcus agalactiae	*Staphylococcus saprophyticus*
Capture rate in 10^2 CFU/mL	25 %	52 %

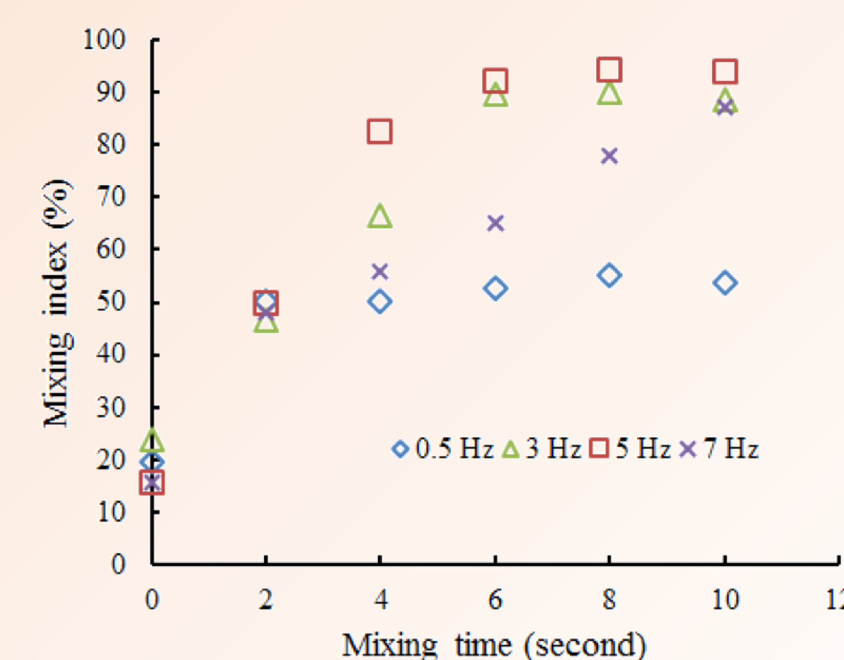


Figure 4: Relationship between the mixing efficiency (index) and the driving frequency of the micromixer. The optimal driving frequency was measured to be 5 Hz since a mixing efficiency higher than 90% could be achieved within 6 s.

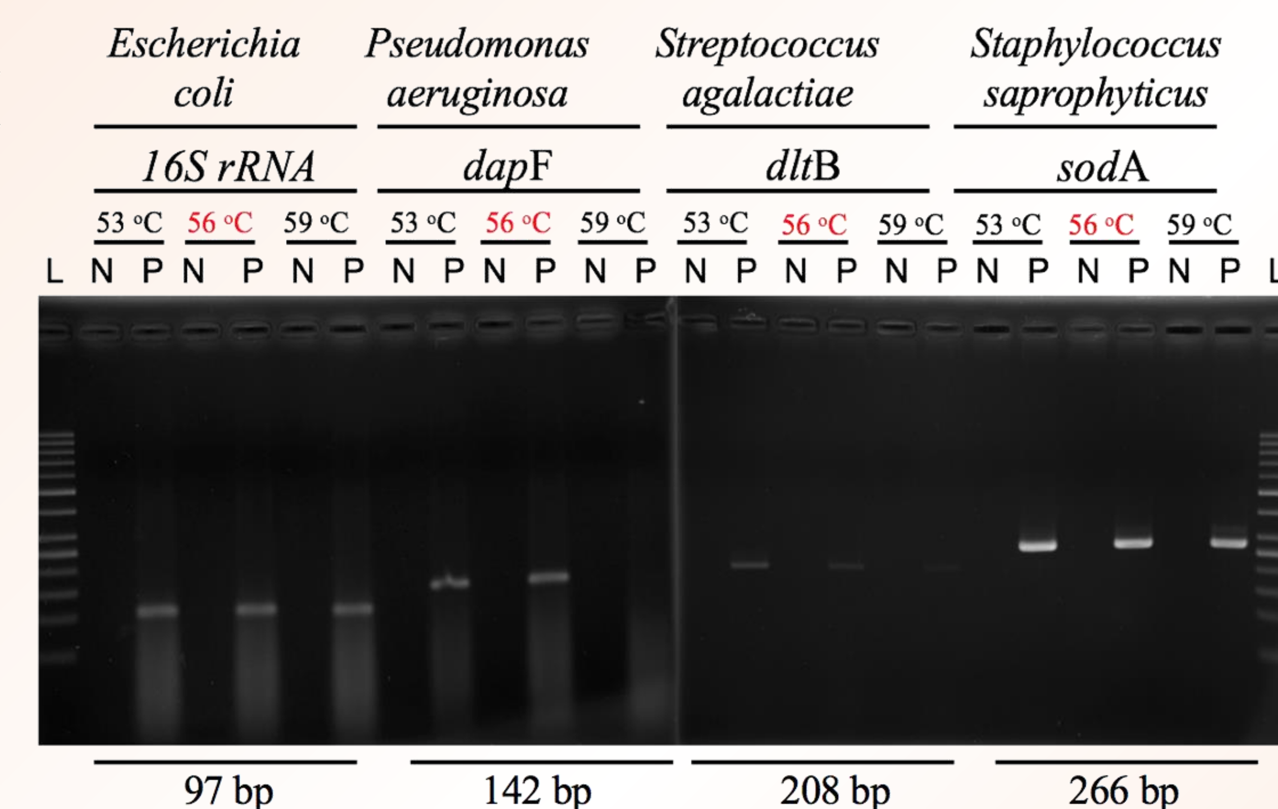


Figure 5: Optimization of PCR conditions for four bacteria. The higher the annealing temperature was, the higher the specificity was required for primers to bind onto targets. Therefore, we chose 56°C rather than 53°C as annealing temperature. (L: 50-bp DNA ladders; N: negative control, ddH2O; P: positive control, bacterial extracted DNA)

Conclusions

- ✓ The entire process including sample pretreatment, bacteria capture and PCR identification has been demonstrated.
- ✓ Four bacteria associated with sepsis could be successfully captured d by using FcMBL-coated beads.
- ✓ Optimization of the annealing temperature (56°C) and specificity for PCR amplification of genes for four sepsis bacteria has been demonstrated.