An Automatic Medium Exchanging Microwell Microfluidic Device for SERS-based Antibiotics Susceptibility Test

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ABSTRACT

To ensure appropriate antibiotics treatment, antibiotic susceptibility test (AST) is a common method in clinical therapies for selecting proper antibiotic treatment and preventing misuse or overuse of antibiotics. However, the existing method still undergoes several obstacles, such as time-consuming, label-intensive process and lack of accuracy. Here, we integrate surface-enhanced Raman spectroscopy (SERS) based antibiotic susceptibility test (SERS-AST) and microfluidics system together. According to our current results, we can now achieve the same effect as manual operation using micro-channel automated bacteria washing and achieve the drug sensitivity test of *E. coli* within 4 hours.

Keywords: Surface-enhance Raman scattering, Antibiotic susceptibility test, Microwell, Microfluidics

1. INTRODUCTION

Antibiotic susceptibility test (AST) is a common method in clinical therapies for selecting proper antibiotic treatment and preventing misuse or overuse of antibiotics. Previously, we have demonstrated a surface-enhanced Raman spectroscopy based antibiotic susceptibility test (SERS-AST) for rapid and sensitive bacteria identification from patients with blood stream infections (BSI) [1-3]. Here, we further develop a microfluidic microwell system with the automated and precise fluidic control to automate and address current limitations of SERS-AST, including labor-intensive and prolonged sample preparation processes.

2. METHODS

2.1. The Microwell-SERS system design

The schematic of the microfluidic flow control system is shown in Figure 1A. All fluids in reservoirs flowed through 150-μm capillary tubes guided by 20-μL/80-μL syringe pump, and four automated selector valves. All components are controlled by customized programs written by uProcess software. Three reservoirs are employed, each of which holds a different solution.

2.2. Automatic SERS-AST process

The automated SERS-AST protocol using our microfluidic system is shown in Figure 2. First, a pre-calculated concentration of antibiotics solution is fulfilled and air-dried in each microwell. As the bacteria solution was infused, the antibiotics powder was dissolved and diffused in the microchamber within 20 minutes. After 2-hour bacteria incubation, DI water was introduced to exchange the MHB medium. Such nutrition-insufficient microenvironment would stimulate bacteria to secrete metabolites. Finally, air was flowed into the device to isolate each microwell before attaching the SERS substrate for measurements.

2.3. SERS measurement and spectral processing

The fabrication of the SERS substrate was reported previously [4]. The SERS measurement was performed on a commercial Raman microscope (Horiba Jobin Yvon) and a 632.8-nm HeNe laser as the excitation source. The laser beam was focused by a 50× objective lens to the SERS-active substrate. The scattered radiation was collected by the same objective lens, filtered through a long-pass filter, and sent to an 80-cm spectrograph with CCD for spectral recording. For each sample, the mean SERS spectrum was averaged by four sensing spots under the baseline removal program,.

3. RESULTS

First, 2.0 μm fluorescence beads (5×10^7 beads/mL) were selected to optimize the washing flow rate. The washing flow rate was tuned from 5 to 25 μL/min. Before washing (Figure 3B), the beads concentration is 37 beads/well. Based on the remaining beads number, we found 10 μL/min is the ideal flow rate is 10 μL/min. Next, 10^5-10^9 CFU/mL bacteria were washed by the automated washing process. The SERS signals were measured and compared with the ones of microdroplets under manual washing processes. As shown in Figure 4, the conventional washing showed a higher dynamic range. However, when the bacteria concentration is above 10^7 CFU/mL, its SERS signal can still be distinguished from pure DI water. Finally, the feasibility of using our platform to perform SERS-AST was evaluated. Here, resistant and susceptible *E. coli* at 10^8 CFU/mL were loaded into the microfluidics with or without pretreated 16 μg/mL kanamycin. Then, the buffer washing and bacteria isolation processes were automatically performed by the microfluidic fluidic control system. As shown in Figure 5, the SERS signal of susceptible *E. coli* solution treated with antibiotics showed a much lower signal than the one without treatment, indicating the

suppression of bacteria growth. In contrast, the signal of resistant *E. coli* solution with and without treatment was almost the same. The results showed that our platform can simplify the labor-intensive manipulation processes required in SERS-AST. In summary, an automatic microfluidic system has successfully achieved antibiotic rehydration, culture medium exchange, microwell isolation, and *in situ* SERS measurement. By integrating above features into current SERS-AST, the technique would be more applicable in real clinical applications. Furthermore, the system shows a great potential for multiparallel operations, which can greatly shorten the required diagnostic period for AST and minimal inhibition concentration (MIC).

4. ACKNOWLEDGMENTS

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5. REFERENCES

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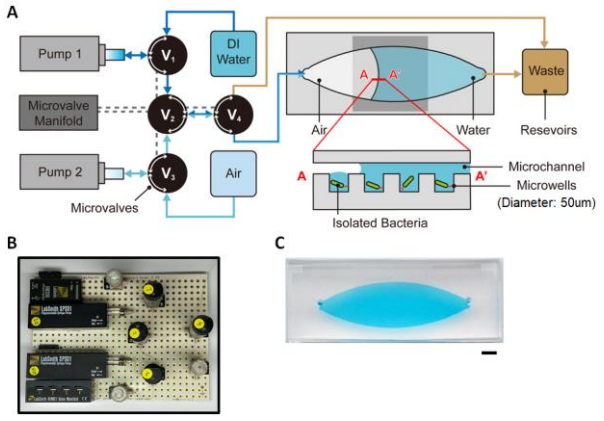

Figure 1. (A) The schematic of the microfluidic device and its flow control system. The photo of (B) the automatic flow control system and (C) the microfluidic device loaded with blue food dye. (Scale bar: 1 mm)

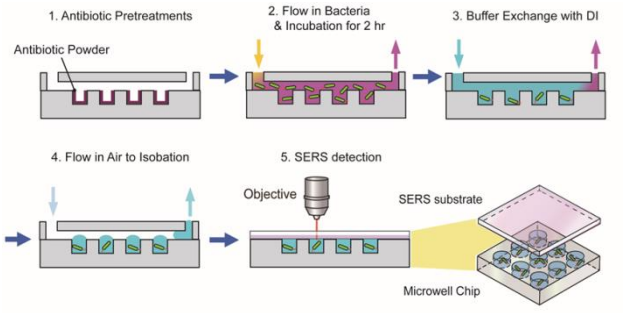

Figure 2. The operation protocol of automatic bacteria antibiotics incubation and culture medium exchange followed by *in situ* SERS measurement.

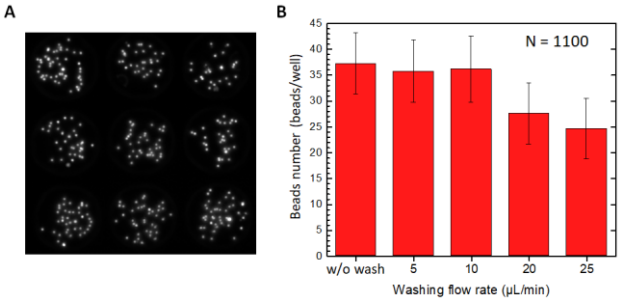

Figure 3. (A) Fluorescent image of beads in the microwells without washing. (B) Fluorescent beads number per microwells after washing (without washing, wash with 5, 10, 20, 25 μL/min, respectively).

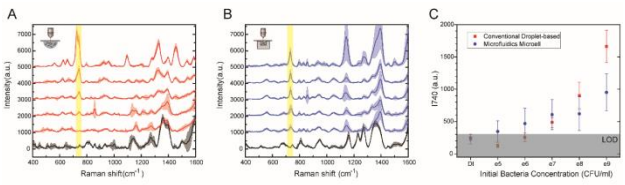

Figure 4. The SERS spectrum of 10^5-10^9 CFU/mL *E. coli* solution using (A) Conventional manual-washed droplet-based method and (B) Microfluidics microwell automatic-washed method on the SERS substrate; (C) A comparison of the Raman signal at 740 cm^{-1} plotted as a function of the bacteria concentration of (A) and (B).

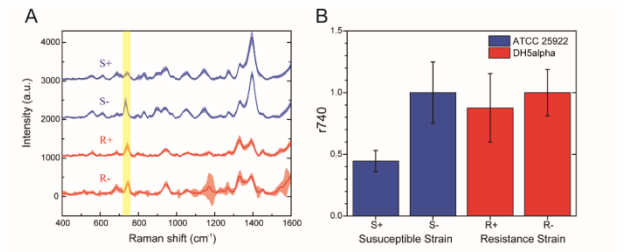

Figure 5. (A) SERS spectrum and (B) r_{740} of bacteria after antibiotic treatment. The blue and red columns are the resistant bacteria strain (DH5-alpha) and the susceptible bacteria strain (ATCC 25922) treated without and with kanamycin, respectively.