A MICROFLUIDIC ANTIBIOTIC CONCENTRATION GRADIENT GENERATOR INTEGRATING SURFACE-ENHANCED RAMAN SPECTROSCOPY FOR MULTIPARALLEL ANTIMICROBIAL SUSCEPTIBILITY TESTING

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ABSTRACT

We developed a microfluidic device with 64 side branch channels and microwell array integrating surface-enhanced Raman spectroscopy (SERS) sensing to perform multiparallel antimicrobial susceptibility testing (AST). The device can generate a stable antibiotic concentration gradient in each side channel based on diffusion behavior in laminar flow. The microwell array underneath each side channel can perform an efficient and uniform bacteria encapsulation and isolation. By replacing the top microchannel by the SERS substrate, an *in situ* and high throughput SERS measurement was done to enable a multiparallel AST. Our preliminary result was using Ampicillin to treat susceptible *E. coli* as a proof-of-concept test.

KEYWORDS: microfluidics, antimicrobial susceptibility testing (AST), surface-enhanced Raman spectroscopy (SERS)

INTRODUCTION

Antibiotic susceptibility test (AST) is a general laboratory procedure to determine the effective antimicrobial to treat bacterial infectious diseases [1]. Although various AST techniques have been widely applied, most methods still required prolonged bacteria culture and labor-intensive sample processes, thereby cannot provide timely clinical decision [2]. To address above issues, we aim to develop a microfluidic system integrating surface-enhanced Raman spectroscopy (SERS) technique to measure bacteria secreted metabolites under antibiotics treatment, as SERS-AST [3]. Compared our previously developed SERS-AST method, in this paper, we propose a microfluidic antibiotic concentration gradient generator to perform simultaneous bacteria treatment under multiple antibiotic dosages, enabling minimum inhibitory concentration (MIC) test in a single device.

EXPERIMENTAL

The microfluidic device consists of the main channel with 64 side channels (top layer) and 12 microwell array underneath each side channel (bottom layer) (Fig. 1A). The assay protocol is shown in Fig. 1C. First, the bacteria solution was introduced to the device (Step 1), followed by antibiotic concentration gradient generation (Step 2) and air introduction to isolate each side channel (Step 3). After 2 hr bacteria incubation, DI water was injected to replace the bacteria broth (Step 4) followed by air introduction to isolate microwell (Step 5). Such buffer replacement creates a nutrient-insufficient environment to stimulate bacteria metabolite secretion under antibiotics treatment. Next, the microchannel was replaced by the SERS substrate for *in situ* SERS measurement (Step 6-7).

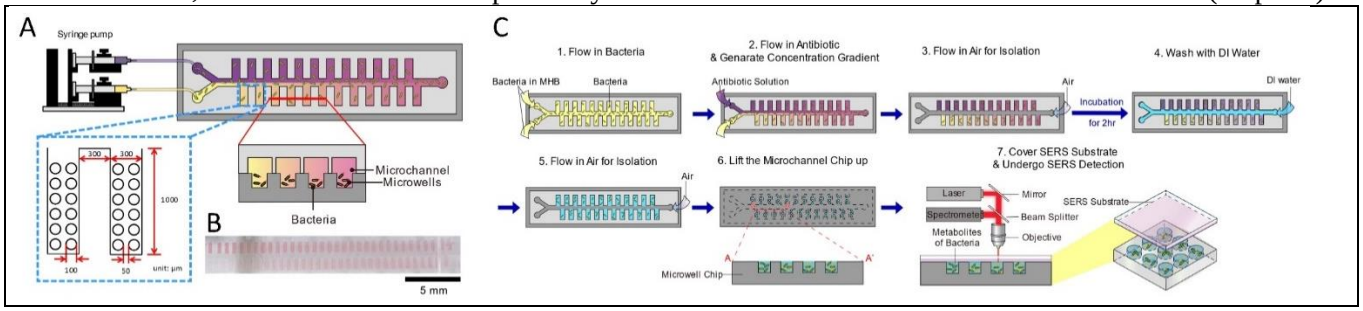

Figure 1: (A) The schematic of the microfluidic device and detailed microwell dimension. (B) The bright-field image of the side channels filled with red food dye with various concentrations. (C) The assay protocol of SERS-AST test.

RESULTS AND DISCUSSION

To find out the ideal flow rate for concentration gradient generation, we perform a COMSOL simulation to evaluate the concentration distribution under different flow rates (Fig. 2A). The result showed that 0.4 μL/min gives the most complete concentration profile (red dots in Fig. 2B). Next, we used this flow rate to introduce Fluorescein isothiocyanate (FITC) solution and water from two inlets to generate a concentration gradient followed by channel and microwell isolation process (Fig. 2C and 2D). The concentration gradient distribution was stabilized in 30 min. Then, the air was introduced from the outlet to isolate each side channel. Finally, the top microchannel was peeled off and replaced by the cover glass for microwell isolation. The averaged fluorescent intensity of 12 microwell in each side channel was plotted in Fig. 2D. The result showed the intensity in channel 25-32 and 57-64 is not stable, which may due to the operational error. Next, we flowed 2 μm Fluoresbrite polychromatic red microspheres (19580-2, Polysciences) to mimic the bacteria loading and encapsulation profile. As shown in Fig. 2E-G, the beads number in the middle of the device (channel 8-24, 40-56) were relatively consistent compared to the sections near the inlets or outlet. We will further optimize the experimental conditions to improve the particle seeding profile. Finally, we conducted a AST using susceptible *E. coli* treated with and without 32 μg/mL Ampicillin for 2 hr. As shown in Fig. 2H and 2I, the SERS signal of *E. coli* treated with Ampicillin indicated a significant lower SERS at 733 cm^{-1} compared to no treated one, representing the lower bacteria-secreted metabolites quantity inhibited by antibiotic treatment [3]. Next, we will generate a concentration gradient of Ampicillin and measure the corresponding SERS signal of bacteria under each microwell with various antibiotic concentration stimulation.

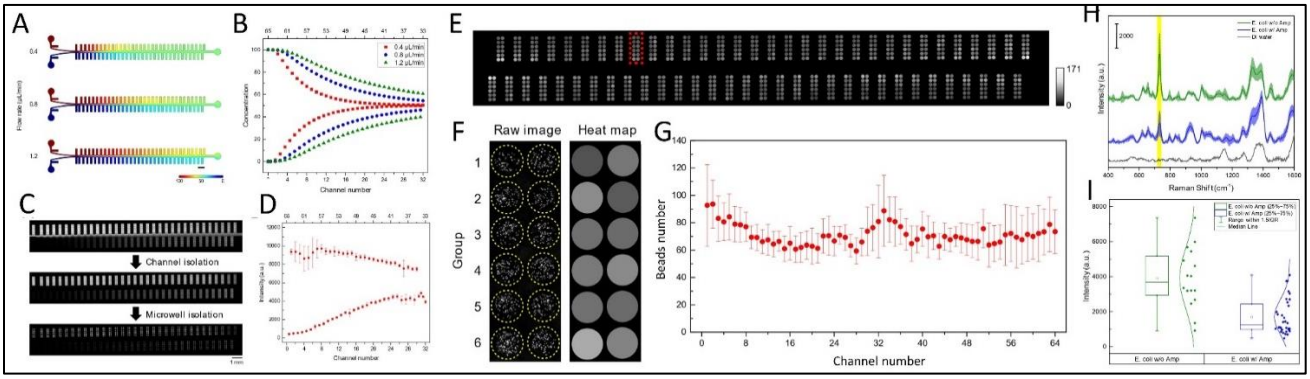

Figure 2: (A, B) COMSOL simulation of microfluidic concentration gradient generation profile under 0.4, 0.8, 1.2 μL/min. (C) The fluorescence images of FITC in the microchannel under (1) concentration generation; (2) channel isolation and (3) microwell isolation state. (D) The averaged fluorescent intensity of 12 microwell in each side channel. (E) The image processed heat map of fluorescence beads encapsulated in the microwell array (F) The raw image and image processed heat map of bead encapsulating in 12 microwell (red box area). (G) The bead quantification results in each side channel. (H, I) Raman spectrum of 10^8 CFU/mL susceptible E. coli treated with and without 32 μg/mL Ampicillin for 2 hours (blue and green line, respectively).

CONCLUSION

We present a microfluidic antibiotic concentration gradient generator integrating SERS to perform multiparallel AST. Our preliminary results have proven the working principle. Next, we will optimize the antibiotic concentration profile, particle encapsulation and introduce different bacteria strains to validate the system performance.

REFERENCES

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