A MICROFLUIDIC DEVICE FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF COMBINED ANTIBIOTICS BY USING BROTH DILUTION METHOD

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ABSTRACT

A simple microfluidic device which can perform antimicrobial susceptibility testing (AST) of combined antibiotics against antibiotics-resistant bacteria has been presented. A new approach to determine the fractional inhibitory concentration (FIC) index of two antibiotics by performing broth dilutions automatically was proposed and its performance was highly consistent with manual operation. The experimental results showed that the developed device could achieve on-chip dispensation/dilution/mixing accurately and interpret AST results visually. A test using two common antibiotics (vancomycin and ceftazidime) was performed on one clinical isolate of vancomycin-intermediate Staphylococcus aureus (VISA) based on the FIC indices. It is the first time that a microfluidic device was demonstrated to be capable of determining the FIC index of combined antibiotics against bacteria by performing AST automatically. With its high flexibility and reliability, the proposed device provides a simple method to explore drug cocktails for combined antibiotic therapy.

INTRODUCTION

As the resistance of bacteria to antibiotics continues to increase whereas the discovery rate of new antibiotics declines, combined antibiotics therapy has been widely practiced to prevent the development of antibiotic resistance and to treat the bacterial infection effectively [1]. However, most of early prescription for severely infected patients was based on personal experience of clinical experts. To address the issue of additional antibiotic resistance and misjudgment, antibiotic susceptibility testing (AST) is a crucial method to determine the proper therapeutic route for accurate diagnosis [2]. Nowadays, the fractional inhibitory concentration (FIC) index is a commonly used formula in AST to provide a quantitative estimate of the activities of two antibiotics in combination against multidrug-resistant bacteria. The current gold standard for determination of the FIC index is conventionally performed by using a checkerboard microtiter plate method. However, it requires a large number of manual steps and time-consuming processes. Furthermore, human error and contamination are inevitable. In order to reduce the assay time and increase operation simplicity and reliability, integrated microfluidics provides an attractive alternative for the multiplexed implementation of AST assays with minimal time and sample/reagent consumption. For instance, a plug-based microfluidic system was reported to confine single bacteria, nutrients, drugs, and fluorescent viability indicators into water-in-oil droplets nanoliters in volume [3]. Similarly, bacteria could be rapidly isolated by using an inertial microfluidic chip to directly determine AST results with a hybridization-based RNA detection approach [4]. Alternatively, cells could be rapidly isolated in microchannels filled with hydrogels, which allowed precise generation of linear gradient of drugs, and the AST results were obtained within 2-3 hours [5]. However, many of these promising routes may suffer from (a) complicated fabrication process, (2) poor portability due to additional large-scale instrument, and (3) unstable droplet formation. Herein, we proposed a simple microfluidic device which can perform AST of combined antibiotics based on broth dilutions automatically to determine the FIC index by visual assessment. With its high flexibility and robust detection results, the proposed device provides a simple method to explore drug cocktails for combined antibiotic therapy.

MATERIALS AND METHODS

Figure 1 reveals a schematic illustration for the experimental procedure of the proposed approach. First, the bacteria suspension, pH-dependent colorimetric broth (BHI media (REF. 237500, BD Co., USA, pH 7.4) supplemented with 1% glucose (G7021, Sigma, USA) and 0.05% phenol red (6455-00, Koch-Light laboratories Ltd, England, pH 7.4)), and two kinds of antibiotics (vancomycin and ceftazidime) (Sigma-Aldrich, USA) were loaded into corresponding chambers (Figure 1(a)). Then, crucial steps including sample dispensation, antibiotics dilution and cell-antibiotics mixing for on-chip AST were performed on the microfluidic chip automatically within 13 minutes. The two antibiotics were diluted to different concentrations and transported to each reaction chamber, respectively (Figure 1(b)). Next, the mixture of antibiotics and bacteria were incubated at 37^{o}C (Figure 1(c)). After 24 hours, bacterial growth was observed while color changes in the pH-dependent colorimetric broth (no growth: red; growth: yellow). The lowest concentration of antibiotics in the combination in which bacteria did not grow was calculated to be the FIC index. (Figure 1(d)).

As top view and an exploded view of the chip were shown in Figure 2, on which the entire procedure was automatically performed. The chip was composed of three layers, including two polydimethylsiloxane (PDMS, Sylgard 184A/B, Sil-More Industrial Ltd., USA) layers and a glass substrate such that it could be integrated with four

transportation units, several normally-close microvalves, and reagent/sample loading chambers. The dimensions of the chip were measured to be 34 mm in width, 56 mm in length and 6.1 mm in height.

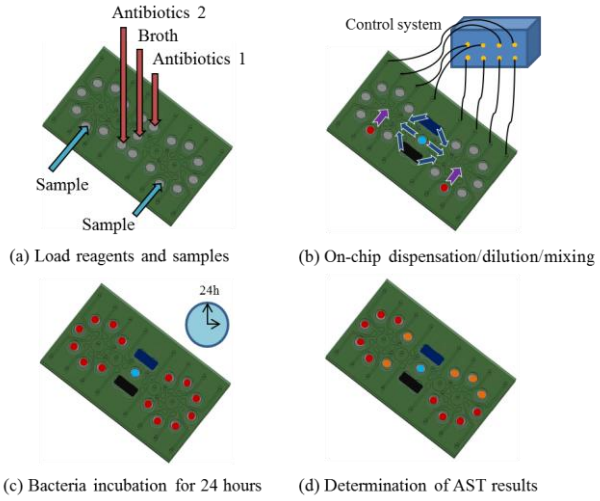

Figure 1: Schematic illustration of the experimental procedure for on-chip AST of combined antibiotics based on broth dilutions. (a) Load the reagents including samples, pH-dependent colorimetric broth, and two antibiotics. (b) Perform on-chip dispensation/dilution/mixing automatically. (c) Incubate bacteria for 24 hours. (d) Determine the FIC index by visual assessment.

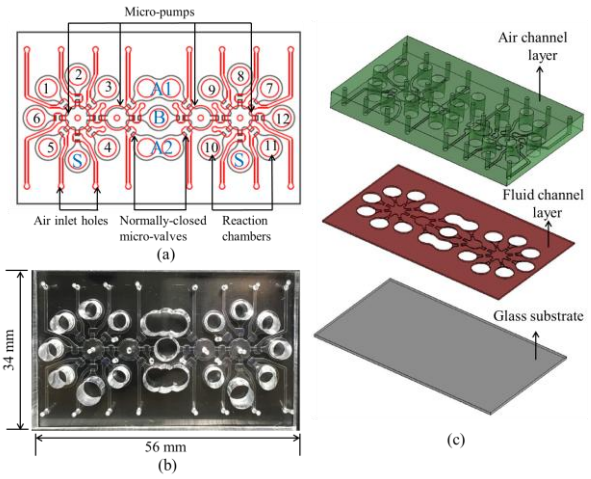

Figure 2: (a) Top view and detailed microfluidic components and (b) a photograph of the microfluidic chip. The dimensions of the chip were measured to be 34 mm in width, 56 mm in length and 6.1 mm in height. (c) An exploded view of the chip which was composed of two PDMS layers and a glass substrate

RESULTS AND DISCUSSION

Characterization of the microfluidic chip

The pumping tests of the micropump were demonstrated in Figure 3. It showed that the uniform pumping volume of each chamber was 27.1 µL in average for broth and two antibiotics. The uniform pumping volume of each chamber represents that the liquid could be transported from reagent chambers to individual chambers with high accuracy. Note that three repeated measurements were performed for each test.

Figure 4 shows the quantitative test of on-chip dilutions for the combination of ssDNA and bovine serum albumin (BSA). The individual concentration of ssDNA and BSA in the mixture (Figures 4(a)&(b)) indicated the reliable reagents dilution of the chip without contamination and were comparable to the manual operation.

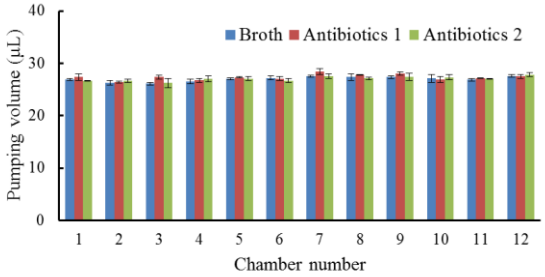

Figure 3: Pumping tests that transported distilled water to chambers 1~12 from reagent chambers of each chamber (n = 3). It showed that the uniform pumping volume of each chamber was 27.1 µL in average for broth and two antibiotics. Note that liquid was transported to each chamber for 6 times and then the volume in each chamber was measured.

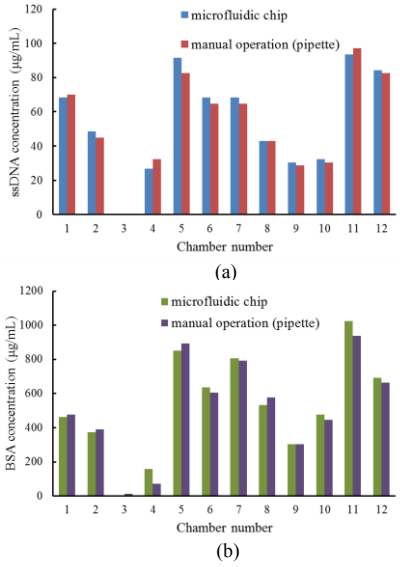

Figure 4: The quantitative tests of the microfluidic chip for on-chip dilution with combined reagents. (a) The comparison of ssDNA dilution in the mixture and (b) the comparison of BSA dilution operation.

Visual assessment of the AST results

Colorimetric results of the on-chip AST of clinical bacteria (VISA; MIC = 3 µg/mL for vancomycin) against concentrations of vancomycin and ceftazidime diluted by the microfluidic device were shown in Figure 5. The incubation times were 0, 10, 17, 24 hours, respectively. The FIC index was calculated to be 4/3 by observing the pH shift-induced color change. Note that the results of three repeated measurements were consistent for the AST.

CONCLUSIONS

A new microfluidic device has been demonstrated in this work that the AST of the bacteria with combined

antibiotics could be performed with customized needs which can provide clinicians to make more precise medical prescriptions. The entire process including sample dispensation, antibiotics dilution and cell-antibiotics mixing for on-chip AST could be performed on the microfluidic chip automatically within 13 minutes**.** A test using two common antibiotics (vancomycin and ceftazidime) was performed by using a clinical isolate VISA bacteria based on the FIC indices. It is the first time that a microfluidic device was demonstrated to be capable of determining the FIC index of combined antibiotics against bacteria automatically.

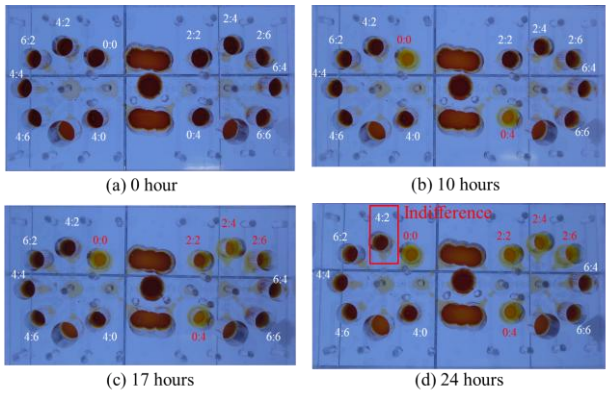

Figure 5: Colorimetric results of AST of clinical bacteria (VISA; MIC = 3 μg/mL for vancomycin) against concentrations of vancomycin and ceftazidime diluted by the microfluidic device. Incubation times were (a) 0, (b) 10, (c) 17, and (d) 24 hours, respectively. Note that the liquid volume of each reaction chamber was about 54.4 μL. The values on the figure represented the concentration of vancomycin and ceftazidime (μg/mL). After incubation at 37°C for 24 hours, the MIC of the combination for vancomycin and ceftazidime were determined to be 4 and 2 μg/mL by the pH shift-induced color change. FIC index was calculated to be $FIC_A + FIC_B = 4/3 + 2/>256 \approx 4/3$.

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