Automated Minimum Inhibitory Concentration System and its Miniaturization.

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Abstract

The purpose of Automated Minimum Inhibitory Concentration System (AMICS) is to develop Minimum Inhibitory Concentration test, known as MIC test, automatically with a more detailed concentration profile. Furthermore, AMICS focuses on the miniaturization of MIC test with same efficiency. To make solutions with diverse concentrations for MIC test, the system generates the concentration gradients by circular and branched channels with serpentine branches between them. Each outlet of the concentration generator makes predictable concentration outcome. The experiments were developed in two ways, one with an adequate meandering structure with no bumps while the other with a shorter meandering structure with bumps in it. The structure with bumps has some advantages e.g., miniaturization of original concentration generator, which is a structure with no bumps, and maintaining similar concentration profile as the original structure.

1. Introduction

When bacteria are exposed to certain amount of antibiotics, normally their growth stops and dies. Therefore finding the minimum inhibitory concentration of antibiotic material is important in that, it prevents giving overdoses of antibiotics to patients and lowers the chance of creating resistance strains. Common method for finding minimum inhibitory concentration, MIC in short, is creating several solutions (about 8 or more), each containing different concentrations of antibiotics, manually. However, the concentration of each solution is usually half of the previous solution meaning that concentrations are sparsely distributed. This leads to only a rough data about the MIC. Also conventional MIC Test requires a lot of solutions, making repeated experiment labor intensive and time consuming. [1]

To make the test simple and fast with detailed concentration profile, efficient concentration generator is needed. Concentration generator is a structure that creates diverse concentration by simply flowing different solutions in its inlets. There are diverse concentration generators e.g., 2D structure that creates concentration gradient purely by diffusion, constantly diluting the solution by introducing buffer as the solution flows by etc. [2], [3], [4] However, none seem to have efficient way of creating and sorting different concentrations created than radial concentration generator. [5] In short, to find more exact MIC value while putting less labor, radial concentration generator was employed.

The radial concentration generator has a lot of meandering structure, so that solution could be well mixed and it can also product diverse solutions with detailed concentrations. However, various meandering structure makes the overall size of the concentration generator big. In this experiment, one structure has the same characteristic of radial concentration generator (Figure 1a) while the other has shorter meandering

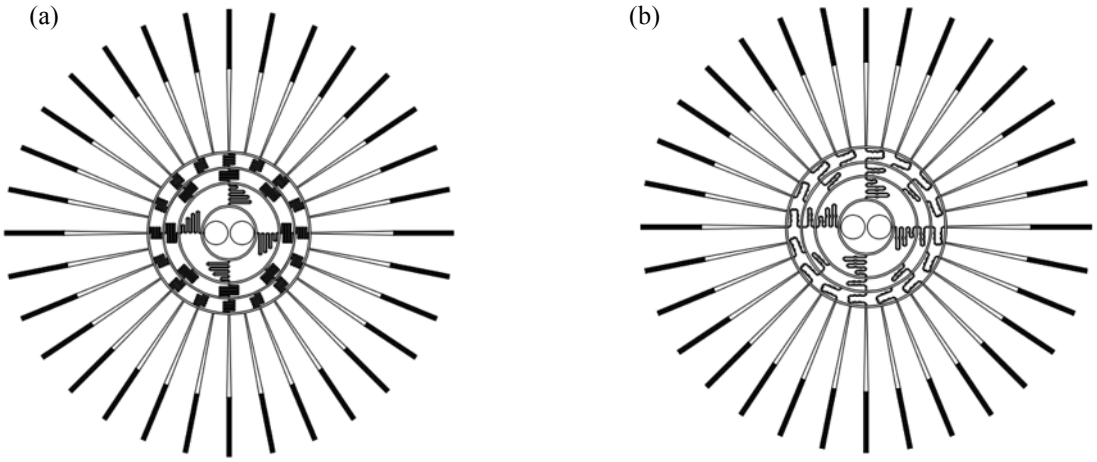

Figure 1 *(a) Schematic of 2-inlet concentration generator with no bump. (b) Schematic of 2-inlet concentration generator with bump*

structure but contains bumps (Figure 1b) that presumably facilitate mixing as two different solutions flow through the structure. [6] This distinct meandering structure is assumed to have similar efficiency in mixing solutions while taking up less space, contributing to miniaturizing the radial concentration generator.

2. Design strategy

2.1 Device design and fabrication

There are multi-circle channels and serpentine branch channels in radial concentration generator. 4 circular channels comprise the multi-circle channels which are positioned concentrically and have 150 ㎛ in width and 40 ㎛ in depth. In case of serpentine branch channels, they are arranged symmetrically around each of the circular channels with 80 ㎛ in width and 40 ㎛ in depth. The inlets (2mm diameter holes) are located in the innermost part of the channel network. The wedge-shaped chambers (7mm in length, 100 ㎛ in width on the narrow side, 360 ㎛ in width on the wider side, 40 ㎛ in depth) are located at the outermost level to capture the bacteria. [5] There are 5 holes (20 ㎛ width) on the end of each chambers where only solutions can pass through.

For the concentration generator described on the Figure 1b, it has same width and depth except its serpentine branches have less curves but contain bumps.

The structures described on the Figure 1 were fabricated in polydimethylsiloxane (PDMS) using soft lithography. In brief, photo curable polymer SU8-2015 was spin-coated on a wafer. After soft baking it was inserted with a mask in photolithography device, and exposed to light. Post exposure bake was carried out after exposure, and then the wafer was immersed in SU-8 developer solution. The process ends by hard baking the developed wafer. The network structure was prepared by pouring the prepolymer of PDMS onto the master. The hardened PDMS bonds permanently to the glass substrate after activated in the air plasma.

2.2 gradient generations

In this experiment we used red and blue food dye both having same concentration. Each dye was collected in 1ml syringe and the syringes were connected to the inlet by tubing. Two different solutions were injected simultaneously into the channel network with same infuse rate by syringe pump. As the solutions flow through the channel network, half of the solution directly entered the branch channel in front of it while each quarter of the solution flowed to adjacent branches. Solutions that moved to adjacent branches met the other solution moving toward it and they were mixed as they passed through the serpentine branch channel. Diffusive mixing occurred, the solution entered the next circular channel and the process is repeated.

The concentration gradient was recorded by a camera mounted on a microscope.

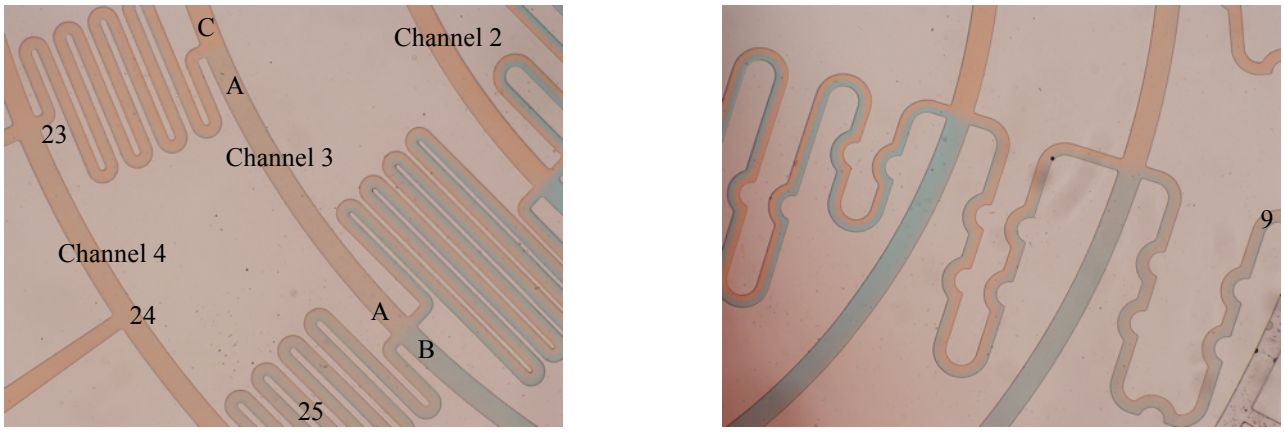

Figure 2 *(a) No Bump experiment 4. This figure indicates that outlet number 23, 24 and 25 worked fine. (b) Bump experiment 1. This figure indicates that outlet number 9 worked fine.*

Table 1 *Result of the Experiments that mixed properly*

Experiments	1	2	3	4
With Bump	**1**, **9**, 11, **17**, **25**	**1**, 5, **9**, **17**, **25**	**1, 9, 17, 25**	**1, 9, 17, 25**
Without Bump	**1**, 2, 3, **9**, **17**, **25**	**1**, **9**, 11, **17**, **25**, 27	**1, 9,** 11, **17, 25**, 29, 30	**1, 9, 17**, 21, 22, 23, 24, **25**

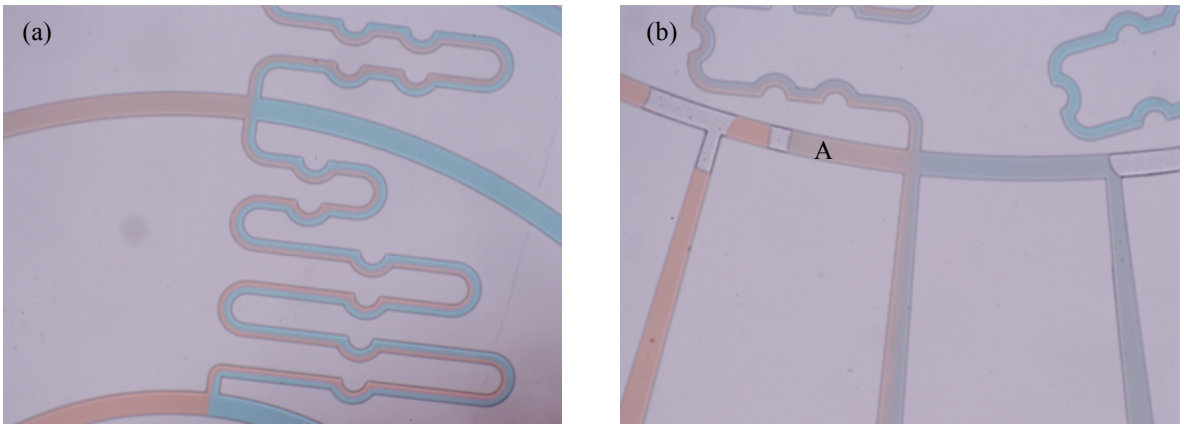

Figure 3 *(a) Not mixed. There is a clear line between blue solution and red solution, which indicates that the two solutions didn't mixed perfectly. (b) Air bubbles inside the channels. The air bubbles block the solutions from mixing each other.*

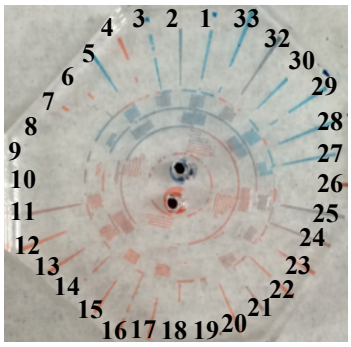

Figure 4 *Full View of concentration generator. The numbers listed on the figure are outlet numbers.*

3. Experimental Results

Table 1 lists the outlet numbers that had mixed properly as expected. Outlet number 1 is pure blue outlet and outlet number 17 is pure red outlet. Outlet number 9 and 25 are the mixture of red and blue with 1:1 ratio. As listed on the Table 1 below, outlet number 1, 9, 17, 25 (marked as a bold text) worked properly as expected on all experiments, Without Bump 1, 2, 3, 4 and With Bump 1, 2, 3, 4. However, other outlets worked improperly or worked only on the particular experiments.

Figure 2a shows that the outlet 23, 24 and 25 made the proper solution for the MIC test. There is a boundary line between solutions A, B and A, C on the Figure 2a, and the boundary lines are heading to the serpentine branch. This implies that the two solutions with different concentrations met at the appropriate spot. After these two solutions pass the serpentine branch, the boundary line had disappeared which means the perfect mixture of two solutions.

Figure 2b implies that the outlet 9 made the proper solution for the MIC test. As the blue and red solutions pass the serpentine branch with bump, it became purple, which is the mixture of red and blue.

4. Discussion

There were two problems on the experiments. First, two different solutions didn't mixed at all after passing the first serpentine branch. To make perfect experiment, two different solutions must become perfectly mixed on the channel 2. However, Figure 3a shows that the two solutions remain almost same on the output of the serpentine branch. Due to this phenomenon, the diversity of the solutions' concentration had disrupted.

Second, the air bubbles inside the channels interrupt the flow of the solutions. Figure 3b shows that a bubble between two solutions interrupts the mixing. Due to the bubble, the solution A on the Figure 3b cannot interact with other solutions around.

There are three solutions to solve these problems. First, the serpentine branch needs more curves and bumps. Suggested number of curves and bumps was not enough to mix the solutions. By adding more curves and bumps, two solutions can be mixed perfectly.

Second, mounting suction on the outlet will prevent the formation of air bubbles. The suction will make pressure difference inside the pipes, which leads to the removal of the air bubbles.

Third, applying adequate pressure will remove the air bubbles in the channels. When the pressure difference is generated across the membrane, gas penetrates the membrane according to the pressure difference resulting the air removal. [7]

5. Conclusion

In this paper we proposed an Automated Minimum Inhibitory Concentration System (AMICS). We suggested two things of the AMICS, automatic concentration generator and its miniaturization. By using this system, MIC test will become fast, simple and precise. However, the proposed system has two problems, improper mixture and formation of air bubbles. Even though the system has problems, there are expected solutions to improve the system. Also, there are numerous other methods to solve these problems such as 'bubble trap'. [8] By solving these problems, AMICS will make various solutions with different and detailed concentrations on a small area. These properties of AMICS will help generating MIC test more convenient and accurate. Moreover, the suggested system can be used on the small chip or machine because of its small size.

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