DROPLET MICROFLUIDIC SYSTEM FOR HIGH-THROUGHPUT SCREENING OF TOXICITY OF ANTIBIOTICS

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ABSTRACT

An automated microfluidic system is capable of forming droplets comprising arbitrary combinations of a number of input solutions [K. Churski, P. Korczyk and P. Garstecki, Lab Chip, 2010, 10, 816-818]. This system can be successfully used to culture bacteria inside the droplets. Addition of a substrate that is turned into fluorescent product via the metabolism of the bacteria allows for monitoring of the toxicity of cocktails of antibiotics.

KEYWORDS: Microfluidics, Automation, Antibiotics

INTRODUCTION

One of primary objectives in the development of microfluidic technologies is to provide tools for research in biology [1]. Low consumption of liquids, speed and ability to provide good statistics all stand in favor of microfluidic systems. Use of droplets as micro-reactors, i.e. droplet microfluidics, is particularly appealing as it offers [2] i) lack of dispersion of time of residence, ii) fast mixing, iii) control over kinetics of reactions, iv) improved statistics through repeated experiments, and v) ultra low use of reagents. On the basis of early demonstrations of a wide range of reactions performed on chip in micro-droplets [2] technologies now appear that offer staggering speed and very low cost, as for example, the recent report on directed evolution of an enzyme [3]. These characteristics (speed, temporal resolution and ability to integrate detection in-situ) have the potential of forming a basis for technology competitive with the microtiter platform. So far, however, ‘digital’ screens of conditions of reactions were possible only in electro-wetting [4] chips and microfluidic systems do not offer the elasticity of commercial platforms.

Here we show an application of a high-throughput droplet screening platform [5] to a study of toxicity of antibiotics and cocktails of antibiotics on bacteria. Interactions between antibiotics are a subject of an increasing interests both from the fundamental and clinical point of view. Interactions reveal the mechanism of action of biologically active compounds [6,7].

EXPERIMENTAL

We designed the layout of the microfluidic chip with the use of MasterCam and than milled the channels in a slab of polycarbonate (Makroclear, Bayer, Germany) with a CNC mill (Ergwind MSG4025, Poland). We then bonded the slab comprising the array of channels with a flat slab of the same material with the use of solvent bonding [8]. The system contained two distinct microfluidic systems – one for generation of the sequence of incubation mixtures that were loaded into a polyethylene tubing for incubation, and one for the readout of the intensity of fluorescence after incubation.

We controlled the flow of the liquids with external electromagnetic valves (V165, Sirai, Italy), modified as described in [5]. In order to ensure on-demand generation of droplets in the range of their volumes between 100 nL and 1 µL, we used the protocol of control of both the continuous and the discontinuous phase [5]. We controlled the valves with standard input-output control cards (National Instruments) and a custom written script in Lab View.

The continuous hydrocarbon liquid was hexadecane and the aqueous streams comprised solutions of tartazine, eosin Y and methylene blue, for the tests of the fidelity of preparing specified concentrations of combinations and solutions of ampicillin, chloramphenicol and tetracycline (Roth, Germany) in growth media (Mueller-Hinton Broth, BD Difco, USA) and a suspension of E. coli in the log phase. In addition, the solutions of antibiotics contained 44 µM (0.0001%) of resazurin (Sigma Aldrich, Germany) that is reduced by metabolizing (living) bacteria to fluorescently active resorufin. We used the ATCC 25922 strain of E. coli and incubated the sequences of mixtures in a tubing (PE60, Becton, US) in a temperature stabilized bath at 37 degrees centigrade for three hours.

For monitoring of the level of viability of the cells exposed to different concentrations of antibiotics we purged the sequence of incubation droplets through a small polycarbonate chip that contained side channels for waveguides. One of the waveguides illuminated the droplets with a wide spectrum light from a mercury lamp, truncated with a short-pass filter. The second waveguide guided the light passing through the droplet to a spectrophotometer (USB4400, OceanOptics). The spectra were subsequently analyzed with a custom written script.

RESULTS AND DISCUSSION

The microfluidic chip (Fig. 1) comprises three T-junction units in parallel. We supply each of these junctions with a continuous hydrocarbon continuous fluid and one of three distinct aqueous solutions. The outlets of these T-junctions merge to a common chamber in which the droplet produced synchronously at each of the T’s merge. In order to ensure coalescence of the droplets into the incubation mixtures we apply an oscillating electric field emitted from an array of electrodes positioned in the vicinity of the merging chamber. The outlet of the chamber leads to a section of meanders that ensure mixing of the final droplets.

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Figure 1: Schematic illustration of the experiment that screens the toxicity of cocktails of antibiotics. Each of the three T-junction units is controlled with two electromagnetic valves to produce a synchronized packet of droplets of specified volumes. These droplets coalesce in the merging chamber and flow through a section of meanders to mix the such-formed incubation mixture (neither the electrodes for electro-coalescence nor the meanders are shown for the sake of clarity). The formed and mixed the incubation droplets are transferred to a section of polyethylene tubing. Once the whole sequence is formed and transferred to the tubing, the tubing is removed and placed in an temperature stabilized bath for three hours. After incubation the droplets are transferred onto a readout plate.

We first used the system to form a sequence of droplets containing all combinations of concentrations of three dyes: tartrazine, eosin Y and methylene blue, having distinct absorption spectra. The scan comprised all possible combinations of the concentrations of these dyes in steps of 10% of the maximum concentration. Figure 2 shows the result of this scan and the fidelity of the automated system for preparation of pre-defined mixtures of input streams.

![Graph showing measured concentrations of three dyes in a sequence of droplets produced in the automated microfluidic system.](image)

Figure 2: The graph shows measured concentrations of three dyes in a sequence of droplets produced in the automated microfluidic system.

We than used the system to prepare incubation mixtures containing a constant concentration of bacteria *E. coli* (~5x10⁶ CFU/ml), a constant concentration of the marker of metabolism (resazurin turned into fluorescently active resoru- fine), and a range of concentrations of ampicilin. This experiment showed that the system can successfully incubate the bacteria and differentiate between the distinct levels of viability of the cells, depending on the concentration of the toxin (Figure 3).

![Micrograph of droplets containing bacteria E. coli, marker of metabolism (resorufin) and different levels of concentration of ampicilin.](image)

Figure 3: Micrograph of droplets containing bacteria E. coli, marker of metabolism (resorufin) and different levels of concentration of ampicilin. Intensity of fluorescence conveys the information about the toxicity of a particular concentration of the antibiotic.
In order to quantify the interaction between two toxins we used the same system to prepare incubation mixtures containing: bacteria, marker of metabolism and a set of concentrations of chloramphenicol and tetracycline. The concentrations of the antibiotics ranged from 0.5 mg/L, corresponding to 10% of the maximum concentration to 5 mg/L (100%). Figure 3 shows three exemplary spectra of intensity of fluorescence recorded from the droplets after incubation of bacteria. The fluorescent signal allows to clearly distinguish between the different levels of viability of the cells subject to different levels of concentration of each of the two toxins. The right panel in Figure 3 shows the normalized intensity of fluorescence recorder from a short sequence of droplets in which the total (combined) concentration of the toxins was fixed to 5 mg/L, and the ratio of concentrations of each of the antibiotics ranged from 9:1 to 1:9. The graph clearly shows a peak of intensity (corresponding to a peak of viability) at a ratio of 1:1, showing an antagonistic interaction between chloramphenicol and tetracycline.

**CONCLUSION**

In summary, the system that we present prepares—in an automated fashion—micro-droplets comprising bacteria, marker of metabolism and a set of toxins. The platform can scan the concentrations of all the constituents at a rate of three droplets per second. The drops are then transferred to a tubing and incubated at 37 °C for three hours and transferred back onto a read-out plate interfaced with a spectrophotometer. Spectra of fluorescence allow for a quantitative analysis of the toxicity of individual compounds and of cocktails of antibiotics.

Proper use of synergism or antagonism between toxins may lead to improved therapies [6,7] that minimize the risk of development of drug resistant strains. Progress in this field, however, critically depends on development of tools for high-throughput assays, as the expense of conventional experimentation prohibits massive screens. As the system that we report can screen up to 10 thousand different combinations of concentrations per hour [5], it should become a useful tool for studies of biochemical interactions in microbiology allowing for extracting detailed maps of interactions in little time.

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**REFERENCES**


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