Surface Energy Trap Assisted Rapid Serial Dilution on Droplet Platform for Bacteria Antibiotics Susceptibility Test

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ABSTRACT

Here we present a surface energy traps (SETs) assisted magnetic droplet actuation platform for complex fluid handling. Dilution series in the form of discrete droplets are rapidly generated on the platform. Bacterial antibiotics susceptibility test is performed in droplets with dilution series of ampicillin generated by SETs.

KEYWORDS:

surface energy trap, droplet microfluidics, bacteria antibiotics susceptibility

INTRODUCTON

Open surface droplet microfluidic platform utilizes discrete droplets as virtual chambers for material containment, exchange and reactions[1-3]. Sample transfers, biochemical reactions and analytes detection are accomplished by dispensing, moving, splitting and merging droplets through a number of actuation mechanisms such as magnetic force[1-3], electrowetting (EWOD)[4], surface acoustic wave[5], and dielectrophoresis[6]. Magnetic droplet actuation has special benefits due to the dual functionality of the magnetic particles (MPs) which serve both as droplet actuator and solid substrate for molecule adsorption[1-3].Magnetic droplet platforms circumvent the need for external fluid control system. A small magnet is sufficient to maneuver the droplets to complete missions. The reduced complexity and full functionality of magnetic droplet platforms make them ideal for miniaturized and portable analysis.

Despite its numerous advantages, magnetic actuation has certain limitations. The major disadvantage is that magnetic droplet is limited to basic operations such as droplet movement, merging and MPs extraction. One very important operation, which is to dispense droplet aliquots from the stock solution, has not been achieved on any magnetic droplet platforms so far.Up to date, only EWOD have demonstrated droplet dispensing. However, EWOD by itself is not able to manipulate magnetic particles which are often required in complex assays as molecule carriers. A secondary mechanism is required to facilitate the particle extraction. Commonly used methods include dielectrophoresis and magnetic force. The additional mechanism further complicates the already convoluted system, rendering it less portable and less desirable for biomolecular sensing.
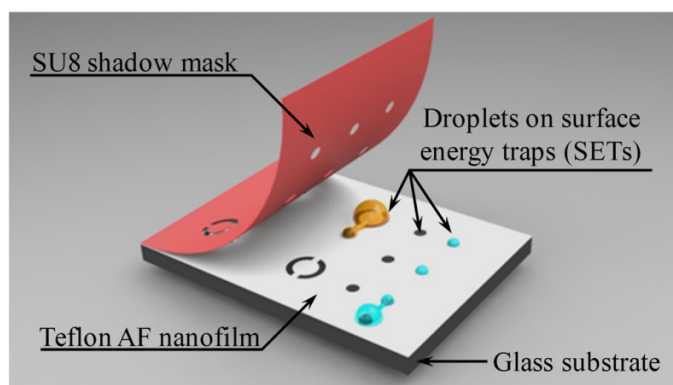

Figure 1: Conceptual illustration of SETs-assisted magnetic droplet manipulation platform. The SETs are patterned by selectively removing portions of the Teflon AF nanofilm using reactive ion etching through a SU8 shadow mask. The SETs-assisted platform allows common droplet operation such as droplet moving and merging. In addition, SETs facilitates particle extraction and enables droplet dispensing, which greatly broadens the applicability of magnetic droplet systems.

EXPERIMENTAL

To overcome the aforementioned issue, we have incorporated SETs to facilitate magnetic droplet manipulation. Depending on the SETs size, droplet volume and MPs amount, SETs can immobilize the entire droplet to assist MPs splitting,

make droplet aliquots and create dilution series (**Fig. 1**). With SETs, the applicability of magnetic droplet microfluidics is considerably extended. SETs are created by etching the Teflon AF nanofilm through an SU8 shadow mask, crafting high-surface-energy islands among lower-surface-energy regions (**Fig. 2A**). They function by altering the surface wetting property. Once the droplet moves over SET, the contact line is pinned down, preventing the droplet from moving (**Fig. 2B-D**). The SET holds the merged droplet in position whereas the MPs plug overcomes the surface tension and splits from the droplet (**Fig. 2B**). In addition, droplets of pre-determined volumes can be metered and aliquoted from the parent droplet using SETs in air (**Fig. 2C**) and in oil (**Fig. 2D**) medium.
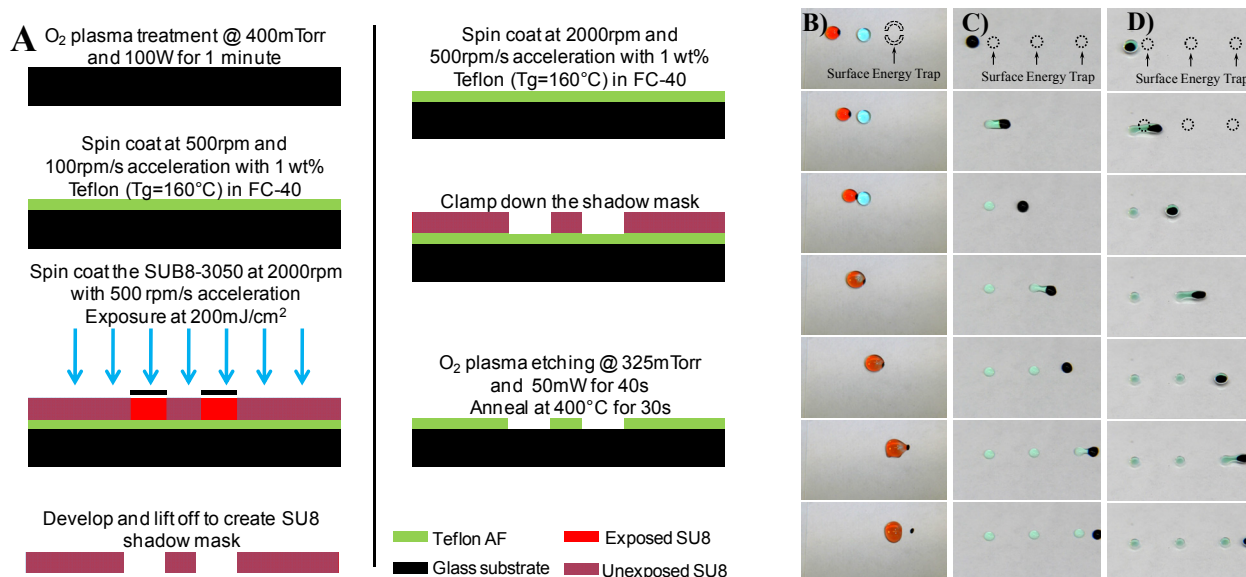

Figure 2: Workflow for creating SU8 shadow and patterning SETs with the SU8 shadow mask. Demonstration of droplet manipulation of the SETs-enabled platform. (A) Droplet moving, merging and particle extraction. B) Droplets dispensing in air. C) Droplet dispensing in oil.

RESULTS AND DISCUSSION

To make dilution series with SETs, the solution buffer droplet is first pulled over an array of SETs (**Fig. 3A**). The array consists of SETs of different sizes which are calculated to create daughter droplets of desired sizes. Then the dilution buffer droplets are dragged to merge with the daughter droplets (**Fig. 3B**). The MPs used to drive the dilution buffer droplets are subsequently removed with the assistance of SETs (**Fig. 3C**). Two dilution series of fluorescein with respective dilution factors of 2 and $10^{1/3}$ are created using water as dilution buffer. The expected concentrations are plotted against the measured concentration in log-log scale. The linear fitting yields slopes of 0.95 (**Fig. 4a**) and 0.9 (**Fig. 4b**) respectively, both of which are close to 1. A slope of 1 indicates the measured concentrations match exactly with expectations.
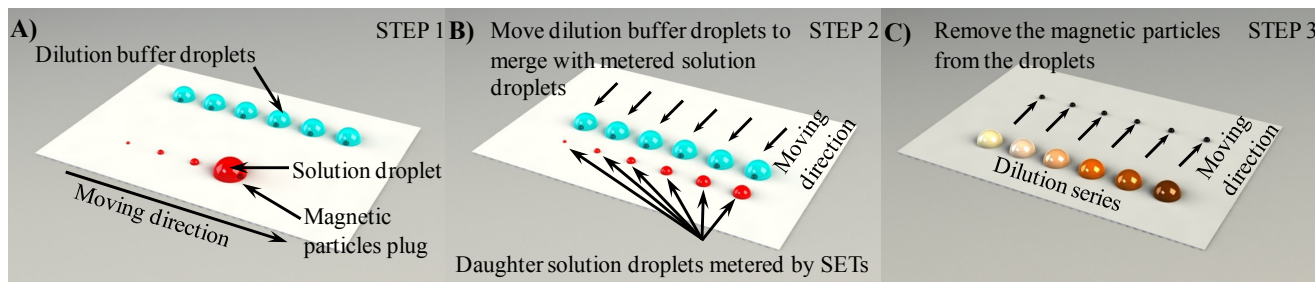

Figure 3: Procedures of making dilution series in droplets with SETs.

A twofold dilution series of ampicillin are generated using this approach. Dilution buffer droplets containing *E.coli* and broth are merged with ampicillin daughter droplets created by SETs. The droplet dilution series are incubated at 37°C for 48 hours in humid chamber. The optical density (OD) of all droplets are measured and normalized to positive control droplet which does not contain ampicillin. The growth of the susceptible *E.coli* strand is inhibited and the inhibition effect becomes more significant with increased antibiotics concentration (**Fig. 5a**), whereas the resistant *E.coli* strand is not affected by the antibiotics (**Fig. 5b**).
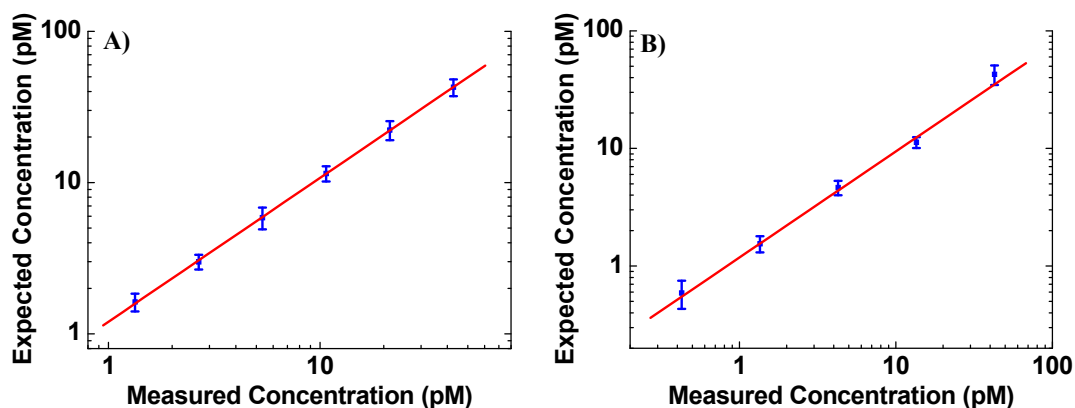
*Figure 4: **A)** SETs meter daughter droplets from the parent droplet to create aliquots. The sizes of the aliquots are uniform with a 3.3% variation in volume. **B)** The volumes of the daughter droplets can be controlled by the sizes of SETs.*
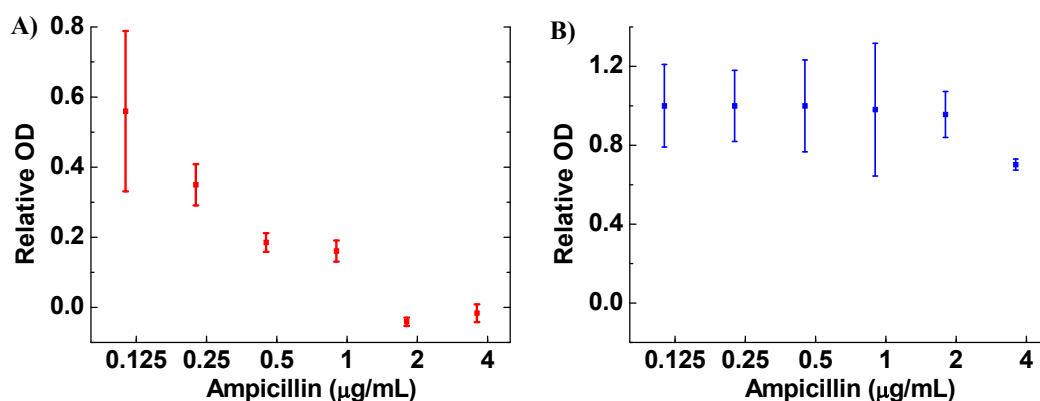

*Figure 5: **A)** Dilution series made by SETs with a dilution factor of 2. The expected concentration is plotted against the measured concentration. The best linear fitting yields a slope of 0.95. **B)** Dilution series with a dilution factor of $10^{1/3}$. The best linear fit has a slope of 0.9.*

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