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Serial Dilution via Surface Energy Trap-Assisted Magnetic Droplet Manipulation

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Abstract

This paper demonstrates a facile method of generating precise serial dilutions in the form of droplets on an open surface platform. The method relies on the use of surface energy traps (SETs), etched areas of high surface energy on a Teflon coated glass substrate, to assist in the magnetic manipulation of droplets to meter and dispense liquid of defined volumes for the preparation of serial dilutions. The volume of the dispensed liquid can be precisely controlled by the size of the SETs, facilitating generation of concentration profiles of high linearity. We have applied this approach to the generation of serial dilutions of antibiotics for anti-microbial susceptibility testing (AST).

Introduction

In the past decade, there has been a growing trend towards downscaling molecular and cellular assays into a microfluidic format to improve assay performance for enhanced throughput, reduced material consumption, and functional integration^{1–6}. While early development of such miniaturized assays was limited to performing only simple fluidic operations, recent advances have greatly extended the applications of microfluidics to assays requiring sophisticated liquid handling. One good example is the innovative microfluidic networks designed to generate serial dilutions and concentration gradients^{7–12}. Serial dilution is one of the most widely utilized laboratory practices. It is an essential and straightforward procedure in many biological and chemical analyses including the characterization of sensitivity and dynamic range of assays, the measurement of reaction kinetic constants, the determination of enzyme activities, and screening the response of cells to drugs and toxins. One popular approach to generating serial dilutions on traditional continuous flow based microfluidic platforms is to control the flow ratio through intricate microfluidic networks^{7, 9, 13–21}. Such designs carefully adjust the flow resistance of microfluidic channels in order to regulate the volumetric flow rate in various branches of the network. Perhaps the most widely adapted design of this kind is the so-called “Christmas Tree” multi-step flow divider developed by the Whitesides group^{9, 18} in which multiple streams of solutions of different concentrations are split and remixed at each level, and the final concentration profile is determined by the mixing levels and splitting ratios.

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More recently, maneuvering droplets on an open surface offers an alternate way of performing bioassays at the micro level^{22–25}. Here, sample droplets are confined by surface tension and function as virtual chambers. In order to actuate these droplets, techniques such as electrowetting on dielectrics (EWOD)^{26–29} and magnetic force^{30–36} have been employed to enable a wide range of droplet operations including dispensing, moving, splitting and mixing^{27, 37, 38}. Yet while droplet microfluidics have been used to facilitate biochemical assays without the need for complex fluidic networks of pumps and valves, they have yet to be amended in order to perform assays requiring serial dilutions. With EWOD, an aliquot droplet can be generated in a fairly straightforward manner. However, the generated volume is often difficult to predict and to control, as it is affected not only by the operation parameters (e.g. driving voltage and signal duration) but also many other parameters (e.g. surface roughness, surface coating and environmental humidity)³⁹. Compared to EWOD, the greatest advantage of magnetic actuation is its capability to simultaneously manipulate liquid and magnetic particles (MPs) for carrying out heterogeneous assays requiring solid phase extraction^{30, 31, 36}. The droplet operations driven by magnets have been extensively studied^{32–34, 36} under various conditions. Nonetheless, traditional magnetic droplet platform has historically been limited to simple fluidic handling. Likewise, operations such as fluid metering and dispensing, which are required for making serial dilutions, are not possible on traditional magnetic droplet platforms. To address this issue, we have recently developed a surface energy trap (SET)-assisted magnetic droplet manipulation technique³⁷ (Fig. 1). SETs provide an additional mechanism for droplet control, enabling comprehensive, magnetically actuated fluidic operations for complex bioassays. We have previously explained the underlying working principle of SETs, and have demonstrated multiplexed sample-to-answer genetic analysis on a SET-assisted platform³⁷.

In this report, we present a magnetic droplet microfluidic platform capable of facile and rapid generation of serial dilutions enabled by SETs that meter and dispense fluid at defined volumes. We then demonstrate an antibiotic susceptibility test (AST) using the developed platform by preparing droplet-based serial dilutions of antibiotics using the SET method.

Results and Discussion

SETs are regions with high surface energy, such as bare glass, surrounded by a substrate with low surface energy, such as patterned Teflon AF thin films (Fig. 1). The device (Fig. 1b–d) is fabricated using the protocol previously described³⁷. To briefly recap, a shadow mask is first made out of SU8 photoresist using a lift-off process. The shadow mask is then laminated onto the glass coverslip coated with a thin film of Teflon AF. After being subjected to reactive ion etching, the Teflon film is annealed at elevated temperature.

The process of magnetic manipulation of droplets assisted by SETs is shown in Figure 2. Droplets are dragged by MPs which are in turn controlled by a permanent magnet situated beneath the glass substrate. The Permanent magnet is either operated manually or *via* a 2-axis motorized stage. The regions enclosed by the dash lines (Fig. 2a–c) indicate SETs, whereas the rest regions are coated with Teflon AF. Droplets are able to move freely and merge with other droplets on the surface with low free energy (Fig. 2a and supplementary information Video V1). In Figure 2b (supplementary information Video V2), the large surface tension provided by the SETs stops the droplet from travelling along with the MPs, which facilitates the extraction of MPs from the droplet. In certain cases, SETs are too small and cannot immobilize the entire droplet. Instead, only a small portion of the liquid is held back within the boundary of the SET (Fig. 2c and supplementary information Video V3). This mechanism can thus be used for liquid metering and dispensing.

Daughter droplets dispensed using SETs display high uniformity. We estimated the volume of droplets by first dispensing droplets containing 100 nM fluorescein using SETs. 10 μ L of water was then added to the daughter droplets and thoroughly mixed. After that, the fluorescent intensity was measured using Nanodrop ND3300 fluorospectrometer. The volume was calculated based on the change in fluorescent intensity using Equation 1:

$$V = \frac{I_f}{I_i - I_f} V_{H_2O} \quad (1)$$

where I_i and I_f are the fluorescent intensities of daughter droplets before and after water is added, respectively. The fluorescent intensity is proportional to the concentration of fluorescein (see supplementary Figure S1). V is the volume of the daughter droplet and V_{H_2O} is the volume of water added.

Based on the initial results, the volume of the daughter droplets could be defined by designing the size of the SETs corresponding to the respective appropriate volume (Fig. 2d). By approximating the daughter droplets as a spherical cap, the volume of the daughter droplet V

$$\log V = 3 \log r + f(\theta) \quad (2)$$

where r is the radius of the SET and θ is the contact angle (see supplementary information for deviation). With constant contact angle θ , the linear regression in the semi-logarithm plot should have a slope of 3. In our case, the slope equals 3.2 which agrees reasonably well with the predicted value. The difference may result from the contact angle hysteresis. The ability of SETs to create sub-microliter droplets provides a rapid and reliable way of creating droplet microarrays on a magnet-driven droplet microfluidic platform (Fig. 2e).

SETs provide a new strategy for creating serial dilutions in the form of droplets because they are able to dispense droplets of defined volumes (Fig. 3a and supplementary information Video V4). To create the serial dilution, an array of SETs of increasing sizes was fabricated, where the diameter of each SET was calculated based on the dilution factor and the volume of the dilution droplets according to Equation 2.

$$V_{n+1} = \frac{V_w}{m_1 D^n + D^n - 1} \quad (3)$$

where V_{n+1} and V_w are the volumes of the $(n+1)^{th}$ droplet and the dilution buffer droplet, respectively. The I^{st} droplet is defined as the one with highest concentration in the serial dilution. D is the dilution factor defined as C_{n+1}/C_n , where C_{n+1} and C_n are the concentration in the $(n+1)^{th}$ and n^{th} droplet. m_1 is the volume ratio of the dilution buffer droplet to the I^{st} droplet (see supplementary information for derivation).

The calculated droplet volume was subsequently mapped to the size of the SETs using a calibration curve (Fig. 2d). A single droplet containing stock solution was dragged over the SETs, which dispensed daughter droplets of defined volume as dictated by the size of each SET (Fig. 3a and supplementary information Video V4). Equal-volume droplets made of dilution buffer were then moved from the Teflon surface to merge with the metered stock solution. Finally, the MPs were removed, leaving only a dilution series in microliter volumes. Despite of the small volume of the daughter droplets, and the entire dilution process completes within a few seconds (see supplementary information Video V4) during which the evaporation is negligible. We demonstrated the serial dilution by using a SET

device to create a dilution series of fluorescein with a dilution factor of 2. The measured fluorescein concentrations were plotted against the expected concentrations, which resulted in a highly linear correlation with a slope of 0.95 (Fig. 3b).

Because dispensing liquid using SETs can only be achieved under certain conditions determined by amount of MPs, the size of SETs, and the volume of the parent droplets³², which limits the volume of the daughter droplets and in turn results in a relatively narrow dilution range of about two orders of magnitude. Therefore, the SETs based serial dilution is well suited for applications requiring small dilution factors such as ELISA and AST. We demonstrated the SET chip for AST by assessing the susceptibility of *Escherichia Coli* to ampicillin using a broth dilution test⁴⁰. Two strains of *Escherichia Coli* samples were isolated from patients and their susceptibilities to ampicillin were determined using standard bench-top method. The two strains, one resistant to ampicillin and the other susceptible to ampicillin, were inoculated into LB broth and grown to about 0.2 optical density (OD) and then diluted by 10-fold. A two-fold dilution series was created from ampicillin stock solution using SETs. 10 μ L of diluted *Escherichia coli* in LB broth was used as the dilution buffer. The two strains of *Escherichia coli* were separately cultured in ampicillin-containing droplets. Two control droplets without ampicillin, each of which contains one of the aforementioned *Escherichia coli* strains, were included. After 24 hr incubation in a humid chamber, which prevents evaporation, at 37°C, the OD of the droplets was measured using a NanoDrop 2000UV-Vis spectrometer. The ODs of the samples were normalized to the respective control. The final bacteria densities were then measured and plotted against the ampicillin concentration. The resistant strain was not affected by the antibiotics, and showed high growth rate regardless the ampicillin concentration (Fig. 3c). In contrast, the susceptible strain had slowed bacterial growth, with a minimum inhibitory concentration of approximately 2 μ g/mL. The results from the SET platform agreed well with bench-top experiments in which the dilution series and bacterial culture were performed in a tube (supplementary information Fig. S2).

Conclusion

Currently, a typical bench-top assay requires fluidic operations which involve removing, transferring, mixing, metering, dispensing liquids, and the combination of these fluidic operations for more convoluted tasks such as serial dilution. SETs translate all of the liquid operations required for assay preparation into the droplet format, thus allowing complex tasks with microliter volumes, and providing a versatile system for portable molecular sensing. With the assistance of SETs, we have developed a simple method of preparing serial dilutions on an open-surface droplet microfluidic platform. Such a method exploits the ability of SETs to precisely meter defined volumes of liquid and to generate serial dilutions. In particular, SET-based microfluidic serial dilution significantly reduces the complexity of the system by eliminating the intricate microfluidic network used in continuously flow systems. As a result, SET-based serial dilution offers simpler design, more convenient operation, and greater potential for system integration as compared to conventional microfluidic paradigms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Illustration of SET-enabled magnetic droplet manipulation. (a) Illustration of droplet operations on SETs enabled open-surface platform. SETs control the size of the dispensed daughter droplets. (b) Picture of a SETs device. The patterned circles and rings are regions with SETs. (c) and (d) Side view and bird's eye view of a SETs device.

Fig. 2.

Fluidic operations on a SETs-enabled magnetic droplet microfluidic platform. (a) The red droplet is dragged by the MPs and moves freely on the surface with low surface energy. Once two droplets are brought to proximity, they merge and mix solutions in the two droplets. (b) As the droplet is dragged over the SET by MPs, the droplet is immobilized by the SET. However, MPs break the surface tension and continue travelling. This mechanism is used to assist the extraction of MPs from the droplet. (c) Under certain conditions, instead of halting the entire droplet, SETs only withhold small portion of the liquid, dispensing daughter droplets of defined volumes. (d) The volume of the daughter droplet is determined by the size of SETs. (e) A droplet microarray generated using SETs on a magnetic droplet microfluidic platform.

Fig. 3.

SET-enabled serial dilutions for antibiotic susceptibility tests. (a) Conceptual illustration of making serial dilution with SETs. Step 1: The parent droplet containing stock solution is dragged over an array of SETs of varying sizes to dispense daughter droplets. Step 2: Equal-volume droplets of dilution buffer are moved to merge with the daughter droplets containing defined volumes of stock solution. Step 3: MPs are removed from the dilution series, leaving droplets of different concentration in the serial dilution. (b) The expected concentration of the fluorescein is plotted as a function of the measured concentration in log-log scale. The slope of the linear regression is close to 1, suggesting the actual dilution factor is as expected. (c) Serial dilutions of ampicillin are generated to measure the antibiotic susceptibility of *E. coli*. The growth of the resistant strain is not affected by ampicillin. The growth of the susceptible strain is inhibited by ampicillin with a minimal inhibitory concentration of 2 $\mu\text{g/mL}$.