AUTOMATED SYSTEM FOR RAPID GENERATION AND TRANSPORT OF LIBRARIES OF NANOLITER DROPLETS.

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ABSTRACT

We demonstrate an integrated system (fig.1) for rapid and automated generation of libraries comprising multiple (~10^2-10^3) populations of sub-nanoliter droplets. Each population has a distinct chemical composition. Protocol of generation of libraries that we report comprises i) generation of a sequence of micro-liter droplets by aspiration of small portions of liquid from a 96-well plate ii) injection of this array of droplets into a separate chip, iii) splitting these 'parental' plugs in a flow-focusing (FF) module into thousands of tightly monodisperse daughter droplets of sub-nanoliter or nanoliter volumes and iv) separation of libraries with plugs of a third immiscible liquid. This method is compatible with automated microfluidic systems that generate droplets of preprogrammed compositions [1] and thus bridges the techniques that individually address large (~ μL) droplets with the methods [2] for high-throughput screening of small (~ pL, ~ nL) droplet libraries.

KEYWORDS: microdroplets, splitting, flow-focusing, droplet libraries

INTRODUCTION

Droplet microfluidics can be an interesting alternative to classical robotic stations because they offer lower reaction volumes and higher rate of operations. The challenge for droplet microfluidics is to provide not only throughput but also the flexibility and ability to freely address individual reaction compartments that the microtiter systems offer. The method that we report bridges the automated individual control of the compositions of large (μL) liquid plugs with the techniques for high-throughput screeningof small (pL - nL) drops.

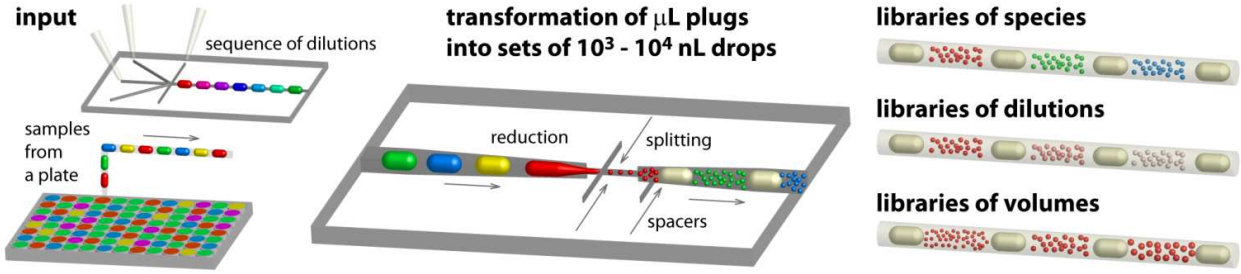

Figure 1: Schematics of the microfluidic system for rapid generation of droplet libraries.

EXPERIMENTAL

Fabrication of the chips. We fabricated the polycarbonate microfluidic chips in a 5-mm thick plates of polycarbonate (Macroclear, Bayer, Germany) using a CNC milling machine (MSG4025, Ergwind, Poland). The milled pieces of polycarbonate were thermally bonded to neat 2-mm plates by compressing them together (30 min, 130 °C, 0.4 MPa). We created the world-to-device interface using 21 gauge needles (~ 4 cm, O.D. 0.82 mm, I.D. 0.65 mm, Fishman Corporation, USA). We connected the device inlets to resistive steel capillaries (O.D. 400 μm, I.D. 205 μm, length 80 cm, Mifam, Poland) extending from the valves using short segments of Tygon® tubing (~ 1 cm, O.D. 0.91 mm, I.D. 0.25 mm, Ismatec, Switzerland) to connect the capillaries with the needles.

PDMS chip were made by polymer casting. The negative masters were made by moulding PDMS (Sylgard 184, Dow Corning, USA) onto PC chip and subsequently silanizing them with vapours of tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane (Alfa Aesar, Germany). Cleaned PDMS negative masters served for moulding of the positive PDMS replicas that were subsequently bonded with smooth slabs of PDMS by exposing both parts to 120 s O_2 plasma and pressing them together. The PDMS chips were subsequently modified hydrophobically by flowing Aquapel into channels (PPG Industries, USA). In order to preserve modification, chip was baked in 70°C for 12 hours.

Optical interrogation of droplets. We used a Nikon SMZ1000 stereoscope coupled with a Photron Fast-Cam 1000k camera to monitor the formation and merging of droplets with the frame rate 18 kfps. We measured the area of each droplet in ImageJ software.

RESULTS AND DISCUSSION

Our system uses a small set of external electromagnetic valves and optical detection [3] and feedback to controllably split and separate the resulting sets of drops with immiscible spacers. We found that in order to minimize the dispersion of the

volumes of these daughter droplets it is necessary to gradually decrease the width of the microfluidic channel upstream of the FF module and that the whole parent droplet must be squeezed into the section of a narrow channel before it enters the orifice of the FF splitter. This procedure omits the problems associated with the changes of the curvature of the parent droplet and thus diminishes influence of Laplacian pressure on the process of formation of daughter droplets (Fig. 2). Another crucial step that ensured production of monodisperse daughter droplets was removal of surfactant from continuous phase of mother droplet stream.

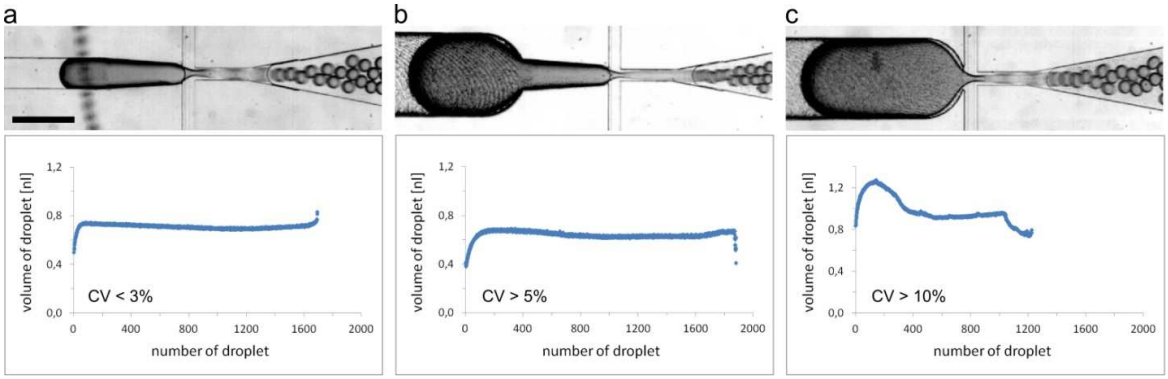

Figure 2. Three consecutive pictures and corresponding graphs show influence of the geometry of the junction on level of dispersity of droplet volume.

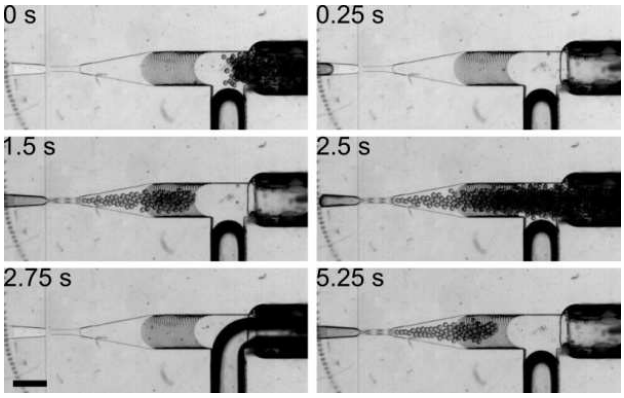

Figure 3. Micrographs illustrating generation of an exemplary library in a polycarbonate chip. Each library is generated with a frequency 0.2 Hz. Each element is generated with frequency ~1 kHz. Libraries are separated by air bubbles generated on demand. Scale bar is 800 μm.

We tested reliability of our system using various oils and surfactants: hexadecane with 3% of Span80; FC-40 and HFE-7500 perfluorinated oils mixed with 3% of triblock PFPE-PEG-PFPE surfactant. We demonstrated that our system is capable of generation of droplet libraries composed of populations of various species (fig. 4a), dilutions (fig. 4b) and volumes (fig. 4b).

We showed preliminary application in testing the viability of bacteria (fig.5) – first we formed library of droplets of LB media containing colonies of E.coli and after 3h incubation in 37^{o}C, we carried out rapid (30 Hz) fluorescence read out. In the close future we will use our system as universal and robust label-free flow cytometer. Stability of the emulsions was provided by PFPE-PEG-PFPE surfactant.

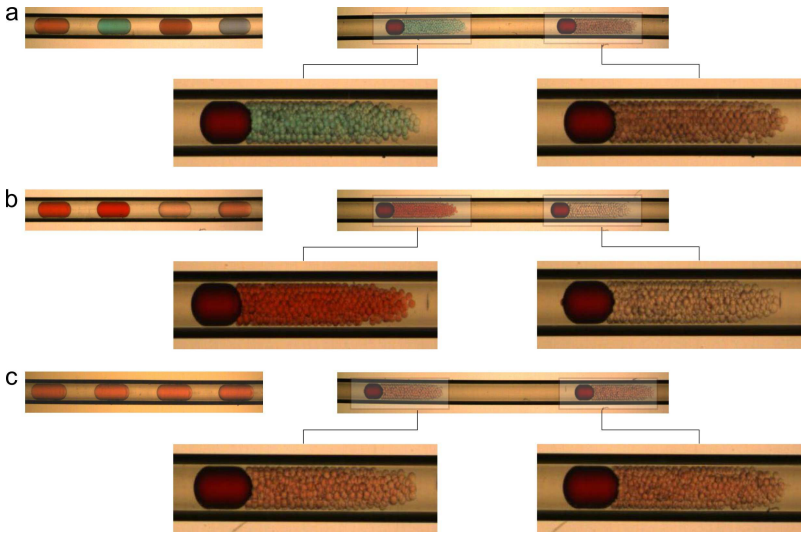

Fig 4. Examples of three different input sequences of parental plugs and the corresponding libraries of daughter droplets. The input sequence was delivered via a I.D. = 0.8 mm FEP tubing. Droplet libraries are separated with immiscible spacers (dyed light mineral oil). Picture a presents libraries with various compositions; picture b – libraries with gradations of concentration and picture c presents libraries with various volumes of daughter droplets.

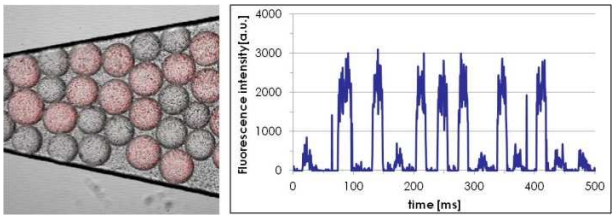

Figure 5. Detection of fluorescence of every droplet inside the library with frequency of 30 Hz. Positive droplet contain bacterial (E. coli) colony and differ in size from negative droplets due to osmosis during 3h incubation in 37° C.

CONCLUSION

Here we present a microfluidic system for rapid and automated generation of droplet libraries. Our technique uses micro-liter plugs of preprogrammed compositions and splits them into ensembles of nL droplets. As such, this method bridges the ability to program the compositions of micro-liter volumes with the high-throughput screening devices. The method that we describe can be useful in exploiting the synergy between individual control of the compositions and high-throughput studies of single cells or molecules and in digital droplet PCR systems.

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