STIRRING IMMISCIBLE LIQUIDS IN NANOLITER CAVITIES

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ABSTRACT

In this paper, we present immiscible liquid mixing within nanoliter droplet-based 'cavities' in microfluidic devices. In doing so, we create biphasic droplets with tunable internal structures, from near-equilibrium drop-in-drop morphologies to complex yet uniform non-equilibrium steady-state structures. The droplets contain an aqueous mixture of poly(ethylene glycol) (PEG) and dextran (DEX), and are injected into an immiscible oil in a simple microfluidic T-junction device. Aqueous droplets with tunable spatial heterogeneity in structure and composition could potentially be used to conduct *in vitro* biochemistry in environments that more closely emulate *in vivo* milieus than conventional macro or micro methods.

KEYWORDS: Droplet microfluidics, Aqueous two-phase systems, Mixing, Biomolecular separation, Chaotic advection

INTRODUCTION

The stirring of viscous, non-diffusing, immiscible fluid mixtures is common to natural and technological phenomena spanning an enormous range of length and time scales; for example, mixing in the earth's mantle due to plate tectonics occurs over thousands of kilometers and several billion years [1] while the blending of immiscible polymer mixtures in static mixers occurs over centimeters and in minutes [2]. Remarkably, simplified models of fluid mixing in lid-driven cavities that account for kinematics of fluid interfaces in steady and time-dependent Stokes flows adequately capture the essential physics involved in these seemingly unrelated phenomena [3]. We demonstrate immiscible fluid mixing at the sub-millimeter and sub-second scale within nanoliter droplet 'cavities' in microfluidic devices. This study enables the creation of droplets with tunable spatial heterogeneity and structure that more closely resemble the complex, crowded and heterogeneous in vivo cellular milieus typical to most natural biochemistry [4, 5]. Further, such droplets possess high internal interfacial area, and can be employed for heat and/or mass transfer in a broad range of biological applications such as extractive bioconversions [6] and biomolecular separations [7].

EXPERIMENTAL

We dispense a phase separating aqueous mixture (a 'Cahn-Hilliard fluid [8]') of mutually incompatible polymers (poly(ethylene glycol) (PEG) and dextran (DEX)) in the form of droplets in an immiscible oil at a microfluidic T-junction (Figure 1a). The microchannels were molded in poly-(dimethylsiloxane) (PDMS) using standard techniques.[9] The relevant microchannel dimensions are width $w = 300$ μm, height $h \sim 130$ μm, and channel length $L \sim 0.36$ m long. There are 11.5 sinusoids in the channel, which began at $x \sim 12$ mm, and the center-to-center distance in each sine curve in the meandering section is ~ 2.8 mm. Syringe pumps (Harvard, PHD 2000) were used to infuse fluorinated oil (FO) (1:10 v/v mixture of perfluorooctanol and octadecafluorodecahydronaphthalene), a solution containing PEG (MW = 8000), and another solution containing DEX (MW = 500 000) to the microfluidic device. The volumetric flow rate ratio of individual aqueous streams, $Q_{PEG}:Q_{DEX}$ was maintained at 1, while that of FO to total aqueous streams, $Q_{FO}:(Q_{PEG}+Q_{DEX})$, was maintained at 1.5. Also, the weight percentage of PEG in the inlet PEG stream was equal to that of DEX in the inlet DEX stream for all experiments. All compositions, *C*, mentioned hereafter therefore refer to the initial compositions of either PEG or DEX in mixture.

RESULTS AND DISCUSSION

At off-critical compositions, the polymer mixture within each droplet spontaneously separates via a spinodal decomposition into two immiscible phases: a PEG-rich outer phase and a dextran-rich inner phase, with very low interfacial tension (in the μN/m range [10, 11]) between phases. For moving drops, in close analogy to lid-driven cavity models of immiscible fluid mixing, the inner dextran-rich (dispersed) phase is 'stirred' within the outer PEG-rich (continuous) phase. The polymer mixture in the aqueous droplets exhibits a near continuum of speed and composition-dependent phase morphologies, from the 'unmixed' static morphology (Lobe 'L') to heterogeneous fragments ('H') and complex percolated morphologies (Reticulate 'R') (Figure 1a(i-iii)).

The formation of these aqueous two-phase systems (ATPS) droplet structures is governed by the competition between the interfacial tension (between PEG-rich phase and DEX-rich phase) and shear stress in the ATPS droplets, which can be characterized by a capillary number, $Ca = U\eta_c/\sigma$ (where U is the flow speed, η_c is the viscosity of the PEG-rich phase and σ is the interfacial tension between PEG and DEX) [12-14]. Increasing compositions lead to increase in interfacial tension [10, 11] and viscosities of each aqueous phase. We construct a dynamic morphology diagram as shown in Figure 1b which maps the influence of flow speed (*U*), and polymer composition (*C*). Surprisingly, the transitions between the different regions on the map may be understood by drawing an analogy with simple models of drop dynamics in unconfined linear Stokes flow

[12-15]. In our case, these models translate to the dynamics of a DEX-rich drop dispersed in a PEG-rich external fluid in linear Stokes flow. In such models, it is well established that a critical capillary number Ca_c exists such that the DEX-rich drop remains stable when $Ca < Ca_c$, and deforms and breaks up when $Ca > Ca_c$; where Ca_c depends on the viscosity ratio and flow type [12-16]. Interestingly, in our experiments, the progression from lobe to heterogeneously fragmented morphologies is well fit by a critical capillary number of 0.25, which also coincides well with previous works in the above model systems [12, 14, 15]. At $Ca/Ca_c > 4$ (curve II in Figure 2), we notice the appearance of reticulate network-like internal structures. The winding microchannels in our device provide time-dependent periodic stretching and folding of fluid interfaces, resulting in chaotic advection of the DEX-rich phase within the PEG-rich phase, similar to that described in the context of miscible fluid mixing [17]. The inner dextran-rich phase is continually stretched, reoriented and folded (Figure 1c); it exists either as extended filaments, or as a heterogeneous dispersion of small drops or filaments in the PEG-rich outer phase. Analysis of high-resolution droplet digital images allows us to determine characteristic sizes of these dispersed fluid elements (D_f), which are found to be in close agreement with calculations based on established 'macroscale' models by Khakhar and Ottino (D_{crit}), as shown in Figure 1d [15, 18].
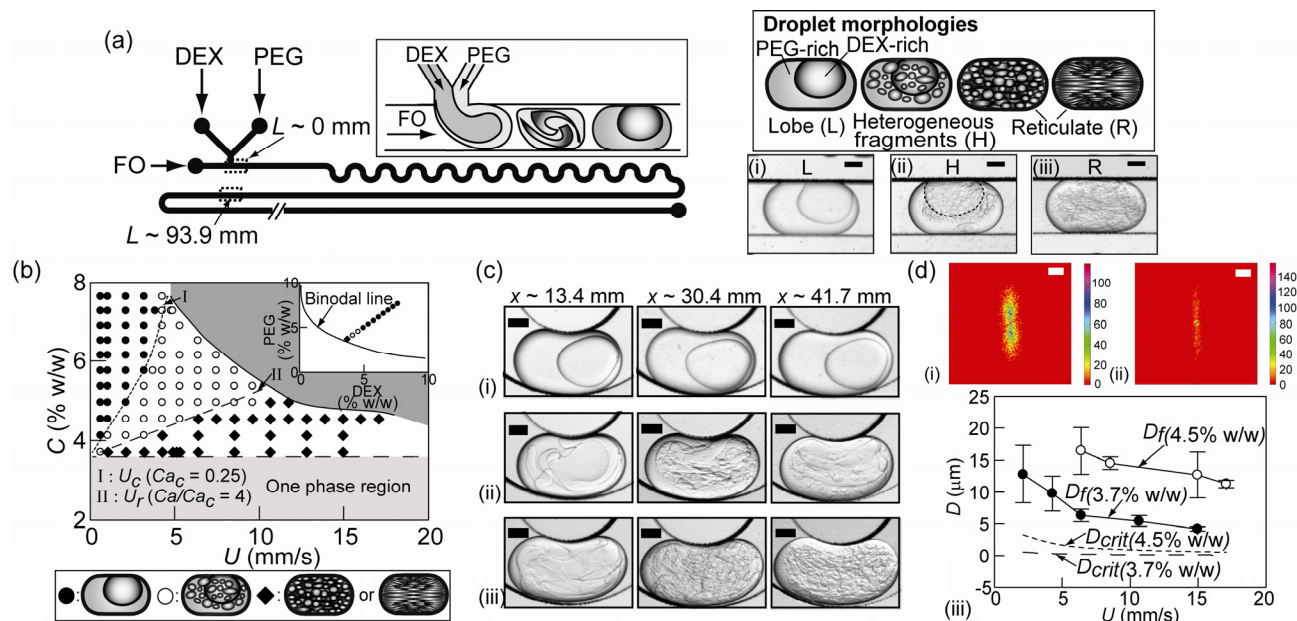

Figure 1: (a) Concept and experimental setup, microscope images captured with varying U (i) 0.64 mm/s. (ii) 4.3 mm/s. (iii) 7.5 mm/s. Scale bars represent 100 µm (b) Dynamic morphology map of PEG-dextran mixtures. Binodal line data are obtained from Diamond and Hsu (1989) [19]. (c) Microscope images obtained along the meandering channel at varying U (i) 0.64 mm/s. (ii) 4.3 mm/s. (iii) 7.5 mm/s. Scale bars represent 100 µm (d) 2D FFT (Finite Fourier Transform) corresponding to (i) 2.1 mm/s. (ii) 15.0 mm/s. (C = 3.7% w/w) Scale bars represent characteristic frequency, f = 20. (iii) Length of filaments, D_f, from FFT with varying U. D_{crit} represent calculated filament sizes.

CONCLUSION

Biochemistry within living cells occurs in sophisticated, spatially structured environments; most *in vitro* biochemical studies in dilute aqueous solutions do not embody this chemical and morphological complexity. Droplet-based aqueous two-phase systems (ATPS) can hence serve as tunable *in vitro* models for *in vivo* cellular environments for a wide array of applications. Moreover, these aqueous two-phase microdroplets possess high interfacial area that can be exploited for a variety of biomolecular applications.

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