MICROFLUIDIC PLATFORMS FOR ON-CHIP FORMULATION AND SMALL-ANGLE X-RAY ANALYSIS OF THE PHASE BEHAVIOR OF LIPID/WATER MIXTURES

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ABSTRACT

We present a microfluidic platform for on-chip formulation and X-ray analysis of lipidic mesophases formed upon mixing lipids and water. The platform is designed to study the effect of detergents on the phase behavior of lipid/water mixtures. The platform allows automated preparation of multiple samples of different composition from stock solutions and subsequent on-chip small-angle X-ray diffraction (SAXS) data collection. To ensure X-ray transparency of the platform we used thin layers of cyclic olefin copolymer (COC) and PDMS. The viability of the platform is demonstrated by mapping out a section of the phase diagram for lipid monoolein mixed with solutions of detergent β-octylglucoside.

KEYWORDS: X-ray transparent chips, SAXS, monoolein, phase diagrams, microfluidics

INTRODUCTION

Upon mixing with aqueous solutions, certain lipids spontaneously form highly ordered liquid crystals also known as lipidic mesophases. Mesophases, especially those of the lipid monoolein, have been used successfully as matrices for crystallization of integral membrane proteins that are otherwise very difficult to crystallize. The key to their success is the fact that their microscopic structure features periodic arrangements of lipid bilayers interpenetrated with aqueous channels. The bilayers maintain a membrane-like environment for the proteins, preventing them from unfolding.

Lipidic mesophases exhibit a variety of phase types, *i.e.*, arrangements of bilayers, depending on the temperature and the composition of the mixtures. The microstructure of a given phase is established using SAXS. The outcome of crystallization trials is dramatically affected by the microstructure of the mesophase, and, in turn, by the components of the crystallization cocktail. Detergents, such as β-octylglucoside, are required to stabilize membrane proteins prior to incorporating them into the mesophase and hence are always present in crystallization mixtures. Due to their amphiphilic properties they have a profound effect on the structure of the mesophase.

The state of the art procedure for the determination of phase diagrams of lipidic mesophases is extremely laborious and consists of preparing each sample on a μL-scale by mixing monoolein and a solution of interest in a coupled syringe mixer, followed by X-ray data collection on samples dispensed into glass capillaries [1].

Here we demonstrate a microfluidic platform that addresses the two most significant challenges associated with on-chip studies of the phase behavior of lipidic mesophases: (1) on-chip formulation of highly viscous lipidic mesophases; and (2) the subsequent on-chip SAXS data collection for phase determination. The design of the chip facilitates handling and mixing the mesophases. Furthermore, the chip is specifically designed to study the effect of detergents on the phase behavior of lipid mesophases. The lack of X-ray transparency of traditional PDMS/glass-based microfluidic devices with integrated fluid handling capabilities is overcome by using only very thin, mostly non-PDMS materials for the chip. We validated the platform by collecting a series of data on mixtures of monoolein with solutions of β-octylglucoside and comparing the results to those obtained with the standard macroscopic method.

EXPERIMENTAL The bulk of PDMS, as well as the glass substrate, were replaced with thin COC sheets.

Chip design and assembly: For the mesophase preparation the chip relied on the lipidic mixer design previously developed in our group [2]. Each sample was prepared in a separate mixer (Figure 1D). Mixing of monoolein with the aqueous solutions to form a mesophase is achieved by a series of actuations of valves located on top of each sample chamber. Hence, the mixer required integrated pneumatic valves and permanent bonding between all layers. Such valves are typically fabricated in multilayer PDMS devices on a glass substrate [3], with the overall thickness of several millimeters, making them unsuitable for X-ray diffraction data collection due to X-ray attenuation. In the hybrid PDMS/COC devices used here only two thin PDMS layers, namely, a control and a fluid layer, were retained to maintain fluid handling and mixing capabilities. These two thin PDMS layers were sandwiched between two COC sheets (Figure 2) to provide rigidity and to eliminate water evaporation from the chip. The overall thickness of the device was ~270 μm, of which ~15 μm is the height of sample compartments (length of X-ray path in the sample). In addition a block of PDMS was bonded to the top COC layer outside of the sample compartment area for world-to-chip connectivity. All layers were permanently bonded to each other: Bonding of COC to PDMS was achieved using amine/epoxy chemistry [4], bonding of PDMS to PDMS followed the standard protocol for multilayer microfluidics [3].

On-chip data acquisition: The small sample thickness (~15 μm) necessitated the use of a synchrotron X-ray source for on-chip SAXS measurements. The measurements were performed on protein crystallography beamline LS-CAT 21ID-D, Advanced Photon Source (APS), Argonne National Lab. The beamline was equipped with microdiffractometer consisting of a goniometer, an XYZ micropositioner, and an on-axis video microscope. For SAXS measurements a vacuum flight tube and an adjustable beam stop with an incorporated pin-diode were installed between the sample and a Rayonix MX-300 detector. We monitored the position of the sample in the X-ray beam using the video microscope (Figure 1C). For sampling various locations within the chip we used the micropositioner to move the chip relative to the 20-40 μm wide X-ray.

Samples were prepared on-chip within 1-4 days before the measurements and stored at -80 ºC to avoid water evaporation. Prior to measurements the samples were defrosted and kept at room temperature (~25±0.5 ºC) for at least 2 hrs.

RESULTS AND DISCUSSION

On-chip sample preparation: Four samples of different compositions could be formulated on chip simultaneously (Figure 1D, E). The number of samples in the chip design was constrained by the overall dimensions of the chip, ~3 x 1.2 cm, which, in turn, was limited by the space constraints of the X-ray beamline. Mesophases were obtained by combining lipid monoolein with aqueous solutions of the detergent β-octylglucoside. The monoolein/aqueous solution ratio was kept constant at 1:1 v/v in all samples, while the detergent concentration in the aqueous solutions was varied. This situation is typical of protein crystallization trials where protein solutions are mixed with monoolein in a constant ratio, but the detergent concentration varies from one batch of protein solution to another. Samples containing different detergent concentrations were prepared on-chip by mixing a concentrated detergent solution and a detergent-free buffer solution in different ratios (Figure 1). Each sample required 9.6 nL of monoolein and 9.8 nL of detergent + buffer solution. A total volume of ~1 μL was required to fill the chip due to dead volume in supply lines and filling ports. For comparison, preparing four samples in the traditional way requires a total of 120 μL of monoolein + detergent solution.

On-chip SAXS measurements: To validate our approach we (i) compared SAXS data collected on-chip with those collected in glass capillaries, the standard method for lipidic mesophases; and (ii) carried out on-chip mapping of a section of the phase diagram for monoolein mixed with solutions of β-octylglucoside of different concentrations.

Figure 3 shows a comparison of X-ray diffraction data on mesophases in a glass capillary and in our microfluidic device. On-chip measurements were carried out using exposure parameters that maximized diffraction intensity while avoiding radiation damage to the sample. The background signal from our hybrid chips was not significantly different from that of thin-walled glass capillaries typically used for SAXS data collection. However, the signal-to-background ratio was substantially lower on-chip than in a capillary due to the large difference in sample thicknesses, ~15 μm vs. >700 μm, respectively. Despite the lower overall signal intensity on-chip compared to samples in capillaries, major reflections (peaks) were clearly resolved, allowing

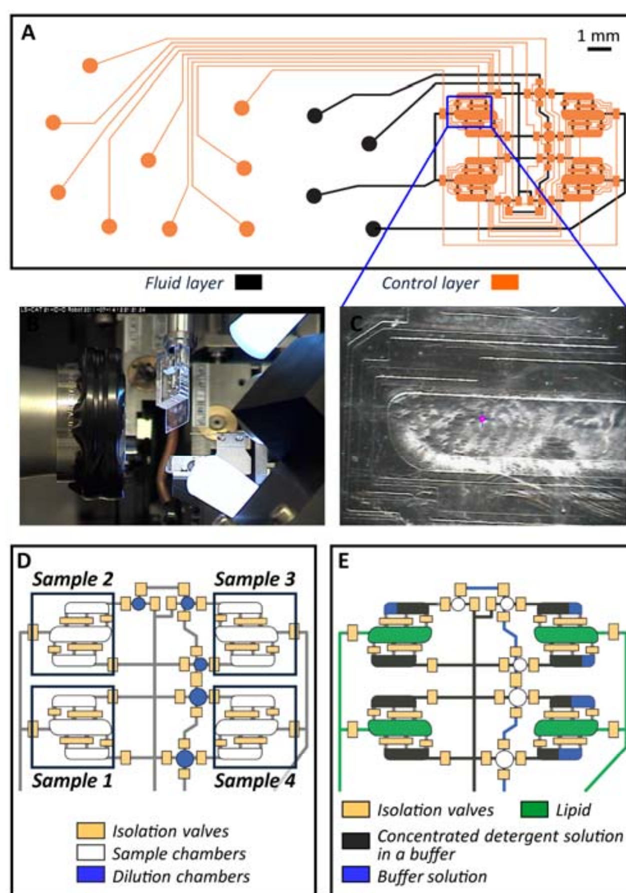

Figure 1: *(A) Schematic of the chip capable of preparing four samples. (B) Chip mounted in the beamline for SAXS data collection. (C) Video microscope view of one sample compartment in the mounted device during SAXS data collection, with the dot indicating the beam position. (D) Four samples of different compositions can be prepared on-chip, with the amount of detergent set by the size of the round compartments (blue). (E) Filled compartments prior to mixing, indicating the relative amount of the three components in each sample.*

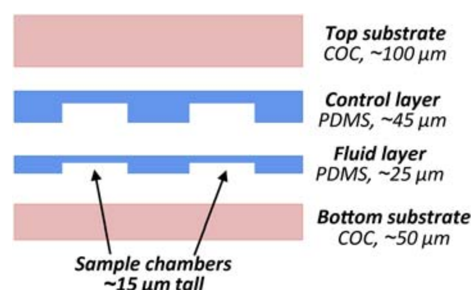

Figure 2: *Schematic of the four-layer chip assembly, comprised of two thin PDMS layers for fluid handling and mixing, sandwiched between two impermeable COC layers.*

the identification of the phase type of the mesophase.

Table 1 summarizes the phase assignments and lattice parameters based on data collected on-chip for several independently prepared samples. The phase types identified on-chip were cubic Ia3d and Pn3m and lamellar Lα, typical for monoolein [1]. Data were obtained by collecting and analyzing diffraction patterns for multiple points within each sample compartment and averaging the results for each type of the mesophase within the sample. Data from different chips were in general agreement with each other with respect to the phase type. The variation in the lattice parameters for a given phase type (Pn3m, Ia3d, Lα) in samples of identical composition between the chips was somewhat higher than that typically observed for comparable samples in capillaries. The same held for the standard deviation in the lattice parameter of the cubic phase (Pn3m) within each sample on-chip. However, overall trends agreed well with previously published data [1]: pure lamellar phases were replaced with co-existing cubic and lamellar phases as the detergent content in the mixture decreased. This confirms the utility of our platform.

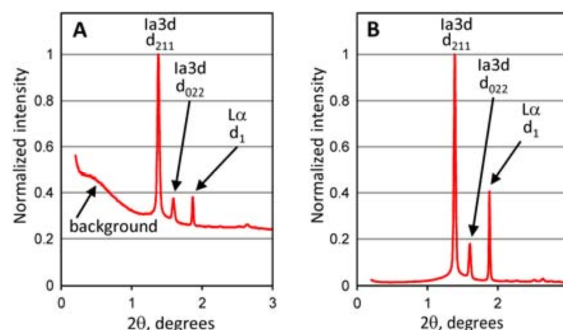

Figure 3: *(A) SAXS data collected on-chip; sample path length ~15 µm. (B) SAXS data collected in a glass capillary, sample path length >700 µm. Ia3d and Lα are the mesophase types identified in the samples.*

Table 1. *Summary of X-ray diffraction data from on-chip measurements.*
Pn3m, Ia3d and Lα are the types of mesophases observed. Values are unit cell sizes in angstroms (Å).[a]

	Sample 1 (20% βOG solution)[b]			Sample 2 (17% βOG solution)[b]			Sample 3 (13% βOG solution)[b]			Sample 4 (10% βOG solution)[b]		
	Pn3m	**Lα**	**Ia3d**	**Pn3m**	**Lα**	**Ia3d**	**Pn3m**	**Lα**	**Ia3d**	**Pn3m**	**Lα**	**Ia3d**
Chip 1	-	49.9	-	-	49.9	-	128 ± 8	49.3	-	158 ± 7	49.9	208
Chip 2	-	49.8	-	-	50.5	-	-	50.4	-	158 ± 8	50.4	205
Chip 3	-	49.9	-	-	50.3	-	145 ± 5	50.2	-	164 ± 5	50.5	-

[a] Lipid: monoolein; buffer: 25 mM NaH_2PO_4, pH 5.5; detergent: β-octylglucoside (βOG). [b] Monoolein/detergent solution ratio 1:1 v/v in all samples. Percentages in parentheses are the concentrations of the detergent solutions (w/v) after dilution on-chip.

CONCLUSION

The platform reported here is a viable alternative to the traditional method of establishing phase diagrams for lipid/solution mixtures. Compared to the conventional approach, a significantly smaller amount of sample is required for mapping phase diagrams of lipidic mesophases and samples of various compositions are prepared automatically. In ongoing work we are using these chips to rapidly determine the phase behavior of a range of lipids to establish their suitability for membrane protein crystallization, especially with respect to their sensitivity to detergent concentration.

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