MICROFLUIDIC PASSIVE PERMEABILITY ASSAY USING ARRAYED DROPLET INTERFACE MEMBRANES

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ABSTRACT

We describe a passive membrane permeability assay using droplet lipid bilayers (DLBs) arrayed in a microfluidic channel. We prepared parallel DLBs between nanoliter-sized donor and acceptor aqueous compartments in lipid oil by coupling microfluidic droplet generation and selective aspiration of excess oil through pseudo-porous sidewalls. We have fluorometrically analyzed the permeation of fluorescein across the DLBs to calculate its permeability. Furthermore, we measured the permeability of caffeine across a DLB by using UV microspectroscopy.

KEYWORDS

Droplet interface bilayers, permeation, diffusion, drug screening, pharmaceutical compound

INTRODUCTION

Membrane permeability assays play an important role in drug discovery to assess passive transport of pharmaceutical compounds across biological membranes. Parallel artificial membrane permeability assays (PAMPA) [1], which use two-layered multi-well plates to measure permeation between top and bottom wells, have been widely used to assess passive permeability of many drug candidates simultaneously. However, the lipid and oil mixtures 10-100 μm in thickness used in this method are dissimilar to biological membranes, leading to longer assay times (many hours) and limiting throughput in measurement.

Recently, several methods have been reported allowing the creation of 2D or 3D networks of droplet lipid bilayers (DLBs) [2–6]; however, no report exists yet on the use of DLBs for quantitative passive permeation assays. Here, we report a passive membrane permeability assay using high-throughput formation of parallel DLBs in a microfluidic channel [7].

EXPERIMENTAL

A wide main channel (width 200 μm) and narrow subchannels (width 20 μm, pitch 50 μm) with a uniform depth of 100 μm were fabricated on a synthetic quartz chip by deep reactive ion etching (DRIE) (Figs. 1ab) and sealed by fusion bonding with another quartz chip. The microfluidic channels were then modified to be hydrophobic by trichloro(octadecyl)silane (Sigma) for both formation of aqueous droplets (Fig. 1c) and selective oil removal (Fig. 1d). The organic phase consisted of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids) phospholipids dissolved in hexadecane to make a 5 mg mL^{-1} solution. For the measurement of fluorescein permeation, we prepared three donor aqueous phases (250 mM fluorescein, 350 mM KCl, 10 mM HEPES) having different pHs (pH 5.4, 6.4, and 7.5). An aqueous buffer without fluorescein (350 mM KCl, 10 mM HEPES, pH 7.5) was used for the acceptor phase. Gastight glass syringes (Hamilton) were filled with those solutions. For infusion and withdrawal of those solutions, we employed three syringe pumps (KDS 220 and Legato 200, KD Scientific). Fluorescein permeation was recorded by a cooled digital CCD camera attached to an inverted fluorescent microscope (Eclipse Ti, Nikon).

RESULTS AND DISCUSSION

Nanoliter-sized acceptor and donor droplets of similar sizes were alternately formed from the two inlets when two aqueous streams were supplied at the same flow rate into the cross-flowing organic stream under low Reynolds and Capillary number condition. Although the generation frequency can be increased as high as 10^3–10^4 Hz at higher flow rates, we typically set low flow rates so as to produce plug-shaped aqueous droplets of 5–6 nL at moderate frequency below 10 Hz (Fig. 2a). This slow generation at low flow rates served to minimize the movement of droplets by residual pressure-driven stream when the infusions by the syringe pumps were stopped for subsequent permeation assay. Also, we set aqueous flow rate Q_w and oil flow rate Q_o to satisfy Q_w/Q_o = 0.1–0.2 so that the prepared droplets can be kept separated from neighboring droplets in the main channel.

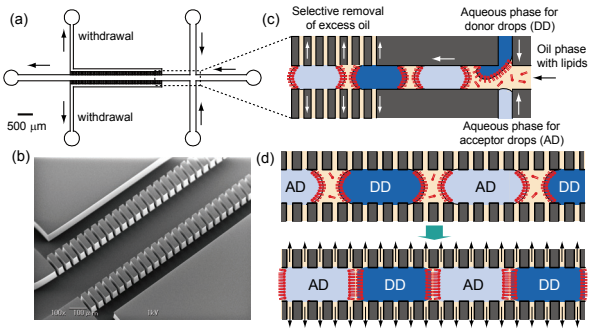

Fig. 1 (a) Schematic of the channel layout comprised of two T-shaped droplet generators and pseudo-porous channel for selective removal of oil phase. (b) A scanning electron microscopy (SEM) image of the main channel (width 200 μm) with side channels (width 20 μm) for the removal of oil. (c,d) Schematic illustrations of the formation and accumulation of aqueous droplets in carrier oil phase for making droplet lipid bilayers (DLBs) between arrayed donor and acceptor droplets (not to scale).

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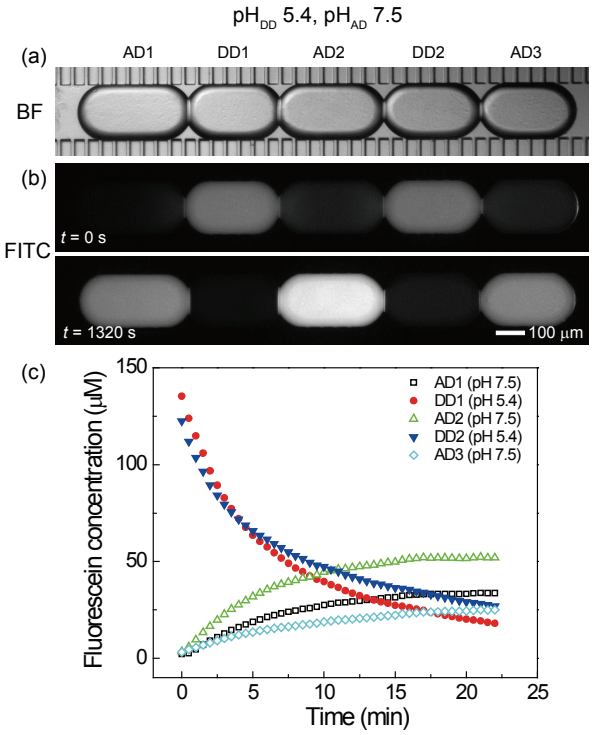

Fig. 2 Measurement of fluorescein permeation in an array of five droplets comprised of two donor droplets (DD) at pH 5.4 and three acceptor droplets (AD) at pH 7.5. (a) A bright field microscopy image of the five droplet array. (b) Time-lapse fluorescent microscopy images at start and end of the measurements (c) Measured variation of fluorescein concentrations in droplets over time.

After the formation of acceptor and donor aqueous droplets at moderate frequency (< 10 Hz), we stopped the infusion of fluids and aspirated excess oil selectively through subchannels to form 1–4 DLBs between 2–5 stationary compartments. Using an array of three acceptors (pH 7.5) and two donors (pH 5.4) having four DLBs (Fig. 2a), we fluorometrically measured the variation of fluorescein concentrations. To do this, we prepared calibration curves that allowed us to map fluorescence intensities to fluorescein concentrations in the droplets. Fluorescein exists in aqueous solutions as either a monocation, an uncharged molecule, a monoanion, or a dianion, and the respective pK_a values are 2.1, 4.4 and 6.4. This indicates that the molecules are predominantly neutral species and monoanions at pH 5.4 and dianions at pH 7.5. It is also known that monoanions and uncharged species contribute considerably to permeation through bilayer membranes. Therefore, the donor droplets act as sources and the acceptor droplets act as sinks for fluorescein. After 20 minutes, the fluorescein concentration of the acceptor droplets significantly increased (Figs. 2b and 2c). In contrast, when donor droplets at pH 7.5 were used, we observed significantly slower permeation (Fig. 3), due to the predominant dianion molecules. Also, in both experiments the fluorescence of the acceptor having two DLBs is noticeably higher than that of the other acceptors with single DLB, indicating the improved permeation through larger membrane area. We

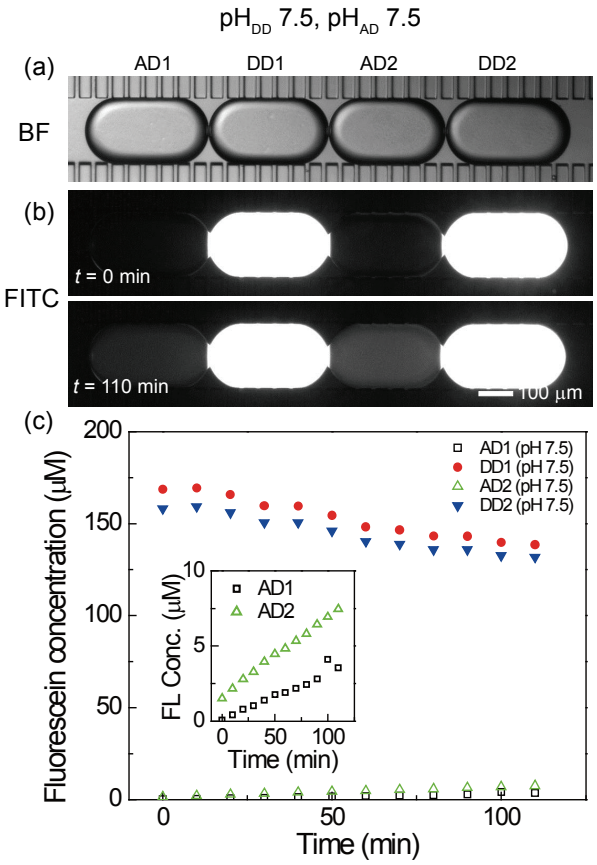

Fig. 3 Measurement of fluorescein permeation in an array of four droplets comprised of two donor droplets (DD) at pH 7.5 and two acceptor droplets (AD) at pH 7.5. (a) A bright field microscopy image of the four droplet array. (b) Time-lapse fluorescent microscopy images at start and end of the measurements (c) Measured variation of fluorescein concentrations in droplets over time. The inset shows the variation of the fluorescein concentrations in two acceptor droplets.

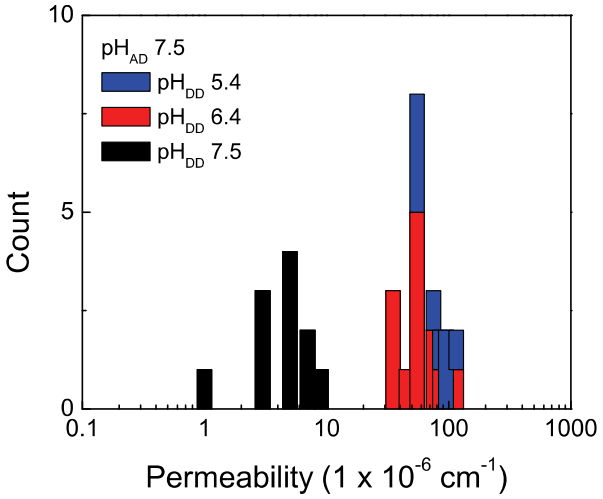

Fig. 4 Distribution of permeability constants obtained from DIBs at three different donor pH values at 5.4, 6.4, and 7.5. Acceptor droplets are always at pH 7.5. Mean permeabilities were 4.7×10^{-6} cm s^{-1} for pH 7.5 (n = 11), 6.0×10^{-5} cm s^{-1} for pH 6.4 (n = 13), and 7.6×10^{-5} cm s^{-1} for pH 5.4 (n = 8).

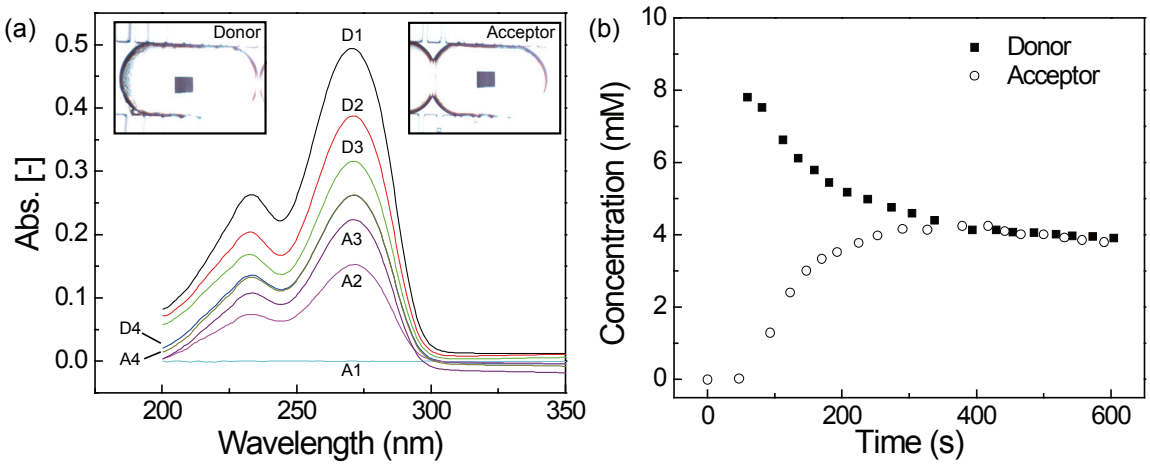

Figure 5. Measurement of a permeability of caffeine through a DIB by UV microspectroscopy. (a) Variation of measured absorbance spectra over time in acceptor (A1–A4) and donor (D1–D4) droplets. A1: t = 0 s, A2: t = 123 s, A3: t = 193 s, A4: t = 327 s. D1: t = 59 s, D2: t = 135 s, D3: t = 238 s, D4: t = 394 s. Inset images are donor and acceptor droplets. Solid squares in the middle of each droplet are the measurement area. (b) Time vs. concentration of caffeine in donor and acceptor droplets.

constructed a permeation model to calculate permeability coefficient for fluorescein of 4.7×10^{-6} cm s^{-1} for pH 7.5 (Fig. 4), which compared well with the published literature[8].

Furthermore, to show the potential of our technique, we determined the lipid membrane permeability of a non-fluorescent pharmaceutical compound by UV-vis microspectroscopy in the confined microchannel. We used a UV-vis microspectrophotometer (20/20 PV^{TM} UV-Visible-NIR microspectrophotometer, Craic Technologies, CA, U.S.A.), which can measure absorbance spectra of the solutions inside the quartz microfluidic chip. We chose caffeine (M_w = 194.19), a well-known drug commonly used as a central nervous system (CNS) stimulant. Caffeine has a pK_a value of 14 in water, indicating that the molecules are predominantly neutral at physiological pH 7.4.

We created a single DLB at the interface between a donor droplet having caffeine (10 mM caffeine, 350 mM KCl, 10 mM HEPES, pH 7.4) of ~5.9 nL and an acceptor droplet without caffeine (350 mM KCl, 10 mM HEPES, pH 7.4) of ~ 4.8 nL. Right after the formation of the DLB, we started to measure the absorbance spectra in the donor and acceptor droplets alternately. We found that the absorbance in the acceptor increased over time, and the absorbance in the donor droplet decreased over time, until the spectra from both droplets became nearly the same (Fig. 5a). This indicates that caffeine molecules passively diffused across the DLB from the donor to acceptor compartment. Figure 5b shows the variation of caffeine concentration over time in the donor and acceptor compartments. The concentration became nearly the same after 6–7 minutes. By using the permeation model described above, we could calculate a permeability of caffeine of ~2.1×10^{-5} cm s^{-1}.

CONCLUSIONS

We have presented a microfluidic platform for a permeation assay using DLBs. In addition to the permeation of fluorophores, the permeation of a non-fluorescent pharmaceutical compound was also measured by UV-vis microspectroscopy in 10–20 minutes. This suggests that our platform can analyze membrane permeabilities of numerous pharmaceutical compounds in significantly shorter assay time than conventional PAMPA. We believe our method could become a basis for a high-throughput permeability assay in drug screening.

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