ACTIVE DRUG LOADING OF MICROFLUIDIC-SYNTHESIZED LIPOSOMES

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ABSTRACT

In this paper, an established method for microfluidic-directed formation of liposomes is combined with in-line sample purification and active drug loading for one-step, continuous flow synthesis of small, nearly monodisperse vesicles containing high concentrations of stably-loaded compounds. Through microfluidic buffer exchange, the presented technique demonstrates the ability to establish a change of 3 or more pH units within 80 seconds, followed by the immediate introduction of a drug analog which is remotely loaded into vesicles at drug:lipid ratios which exceed traditional bulk-scale processes. The result is a "pharmacy-on-a-chip": a microfluidic technique for in-line formation of liposomes containing actively loaded compounds.

KEYWORDS: Liposome; Microdialysis; Remote loading; Ammonium sulfate gradient

INTRODUCTION

Liposomes are lipid bilayer vesicle nanoparticles which have made a remarkable impact as drug delivery vehicles [1]. Although traditional preparation methods result in polydisperse populations of vesicles which require multiple post-processing steps for further reduction in size and size variability, an established microfluidic flow-focusing method provides an alternative strategy for producing populations of small, nearly monodisperse liposomes in a one-step, continuous flow process [2,3].

Despite its appeal, the microfluidic method for liposome synthesis is currently limited to passive drug encapsulation and requires off-chip sample purification to remove residual solvents or non-encapsulated reagents. Active loading, or forced entrapment of amphipathic compounds into the liposome core via ion/pH gradients, enables high concentrations of reagents to be stored within vesicles with excellent long-term stability [4]. This technique, which has proven central to the commercial success of existing liposomal drugs, is currently performed solely by lengthy, bulk-scale processes. Trans-membrane pH gradients are unstable, thus loading efficiency improves the quicker a liposome undergoes external buffer exchange and is introduced to amphipathic compounds [5]. In addition, it has been proven that environmental conditions may be optimized to enhance drug loading efficiencies [6]. In this study, the active loading technique has been implemented within a microfluidic device for rapid buffer exchange and introduction of amphipathic compounds to be remotely loaded. The inherently decreased diffusion lengths in microfluidics enable rapid microdialysis as well as significantly reduced incubation times for drug loading.

This study demonstrates microfluidic buffer exchange and drug loading of an amphipathic drug analog into microfluidic-produced vesicles at high concentrations. The resulting apparatus (Fig. 1) decreases the processing time for active loading from the typical 12 or more hours to less than 1.5 min, enabling exceptionally more rapid and efficient liposomal drug loading than bulk methods.

EXPERIMENTAL

Hybrid PDMS-regenerated cellulose devices were fabricated to carry out this study (Fig. 2). PDMS microchannels were fabricated using soft lithographic techniques. Briefly, dry film photoresist was patterned via UV photolithography on glass slide substrates as used as molds. PDMS was poured onto the dry film photoresist molds and allowed to cure. Inlet holes were created using a biopsy punch. Nanoporous regenerated cellulose membranes were cut to match microchannel patterns using a vinyl cutter and flattened using a hydraulic hot press. PDMS-cellulose hybrid devices were bonded by stamping the PDMS pieces containing microchannel features onto spin-coated PDMS pre-polymer, which were then aligned with the cellulose sandwiched between them, and set in an oven at 70 °C for 3 hours to allow bonding to occur.

For buffer exchange experiments, ammonium sulfate buffer (250 mM, pH 4.6) was injected into the sample channel of the remote loading device and isosmotic HEPES buffer (10 mM HEPES, 140 mM NaCl) was injected into the counterflow. The sample buffer contained pyranine (0.1 mM), a molecular pH
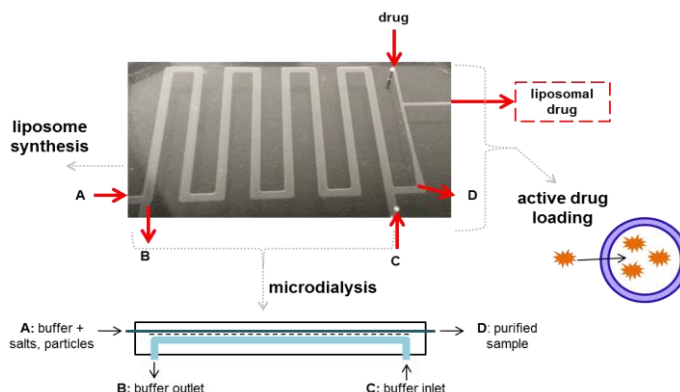

Figure 1. Schematic of the microfluidic device which includes a microdialysis region for rapid buffer exchange followed by a region for active drug entrapment.
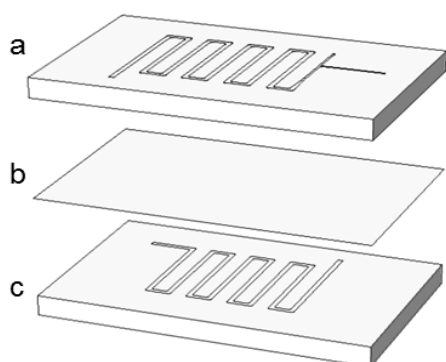
Figure 2. Exploded view schematic of the PDMS/cellulose hybrid microfluidic device with a) sample channel for dialysis and active drug loading; b) regenerated cellulose dialysis membrane; and c) channel for buffer counterflow.

probe, for quantification of pH change of the buffer. The pH change of the sample was investigated for HEPES pH 7.6 and pH 9.6, and under flow velocities ranging from 0.30-0.60 cm/s.

To investigate on-line active drug entrapment, a microfluidic device containing a buffer exchange region with identical dimensions as used previously with an additional downstream region for introduction of additional fluids (Fig.1). Liposomes were synthesized using the microfluidic flow focusing, as described previously [2,3]. Briefly, a center stream composed of an ethanol-dissolved lipid mixture was injected between two sheath flows of aqueous buffer (ammonium sulfate, 250 mM, pH 4.5). The resulting liposomes were characterized for size and used for active loading experiments. Liposomes in ammonium sulfate were injected into the sample inlet of the active loading chip with isosmotic HEPES (pH 7.6) as the counterflow. Acridine orange (AO), a drug analog, was introduced immediately after buffer exchange. Sample velocity and AO concentration were varied to investigate the effect of active drug loading. Resulting liposome populations were placed into mini dialysis units for 4 h to remove remaining external AO. The purified samples were characterized for AO content using absorbance measurements.

RESULTS AND DISCUSSION

The PDMS/cellulose device enabled successful buffer exchange resulting in the sample fluid undergoing a rapid pH change. When the flow velocity was adjusted from 0.6 cm/s to 0.3 cm/s (~7-14 μL/min), the sample buffer pH was rapidly adjusted from an initial value of pH 4.6 to up to 3 pH units in 40–80 s (respectively) (Fig. 3). Microdialysis performance was found to be dependent on flow velocity (residence time), channel height, and counterflow buffer pH to change in sample pH.

In addition to verification of pH change, adequate ion removal was ensured through a numerical simulation of the device (Fig. 4), which verified the extra-liposomal ammonium ion content was <0.001 (a necessary condition for successful remote loading) [4].

Microfluidic-synthesized liposomes were 80.8 ± 17.9 nm in diameter (data not shown). When injected into the sample channel followed by introduction of AO immediately after buffer exchange, drug:lipid molar ratios up to 1.3 were achieved (Fig. 5), exceeding the typical ratios of 0.125 or less achieved by bulk active loading which requires an overnight incubation [7]. Drug loading was not found to be dependent on residence time or flow velocity varied from 0.18-0.45 cm/s (Fig. 5a), but was directly influenced by initial drug:lipid ratio when varied from 0.125–2.5 mol AO/mol lipid. (Fig. 5b).
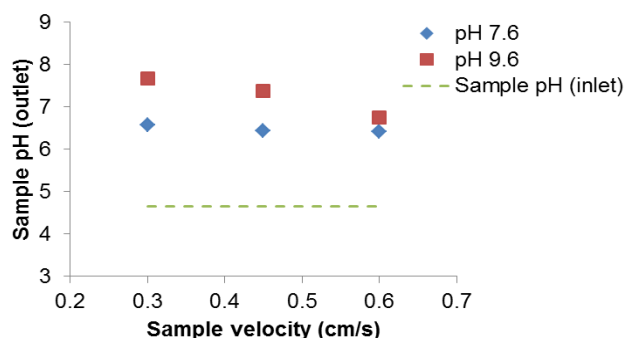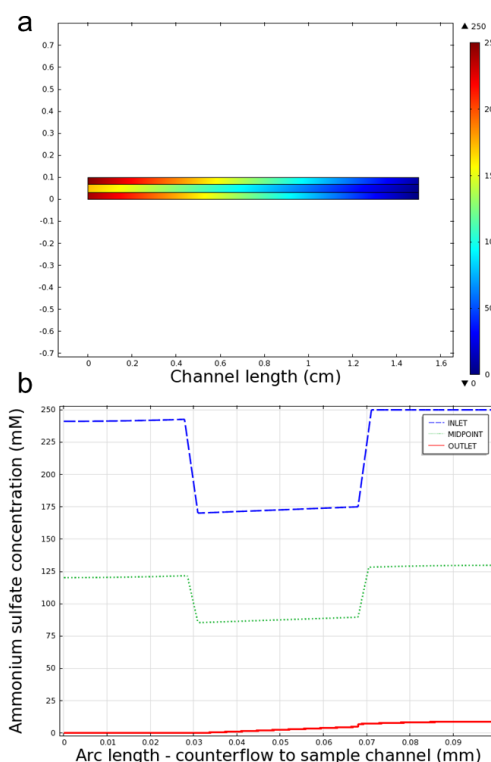

Figure 3. Demonstration of microfluidic buffer exchange at various counterflow pH and flow velocities. Residence times vary 40 s-80 s, ΔpH 1.7-3.0. Total flow rates vary ~7-14 μL/min.

Figure 4. CFD simulations to predict mixing and ion exchange under various flow conditions; a 2D rendering of the concentration profile along the length of the dialysis channel, b) concentration profile at inlet, midpoint, and outlet of the channel, with sections representing the distinct counterflow, membrane, and sample regions (left to right). Example is for 250 mM ammounium sulfate flowing at 0.3 cm/s.

CONCLUSION

Here we present the first demonstration of a microfluidic method for active loading of amphipathic drug compounds into liposomes. The resulting platform represents a comprehensive "pharmacy-on-a-chip" which may be combined with upstream flow focusing for liposome production for realization of a novel one-step, continuous-flow microfluidic assembly line for producing liposomes with high concentrations of stably-encapsulated drugs. This technique may be further investigated to determine optimum conditions for efficient drug loading to produce vesicles with unprecedentedly high concentrations of therapeutic compounds.
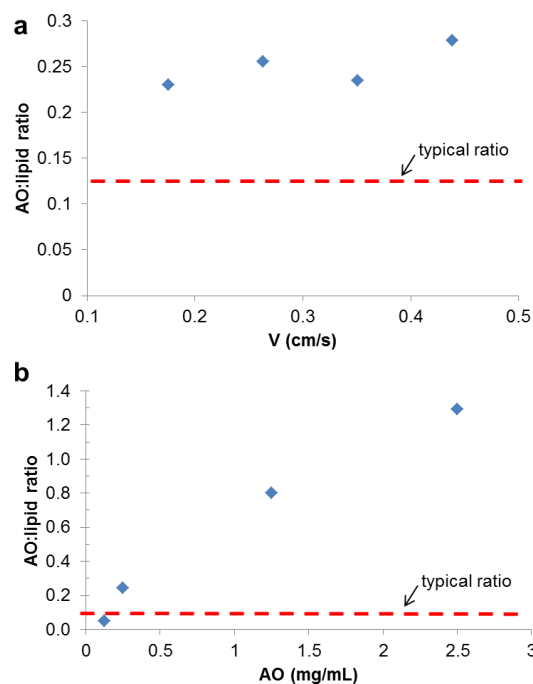

Figure 5. Active loading of acridine orange (AO) dye into liposomes prepared separately via microfluidic flow focusing. The effect of a) sample velocity (AO = 0.25 mg/mL) and b) AO concentration (V = 0.25 cm/s) on AO loading were investigated. Loading drug:lipid ratios of 1.6-2.3 were achieved in seconds, compared to typical ratios around 0.125 (red line) typically achieved through day-long, bulk-scale processes.

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