Colloids and Surfaces B: Biointerfaces 156 (2017) 349–357

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

High-throughput continuous production of liposomes using hydrodynamic flow-focusing microfluidic devices

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ARTICLE INFO

Article history:
Received 14 February 2017
Accepted 10 May 2017
Available online 11 May 2017

Keywords:
β-Carotene
Nanovesicles
Lecithin
Phospholipid

ABSTRACT

The microfluidic hydrodynamic flow-focusing is a simple technique for nanoscale liposome formation that provides several advantages compared to the traditional manufacturing techniques. This work aimed to perform a systematic study of the liposome formation using planar microfluidic devices with different channel aspect-ratios, as an alternative to enhance the throughput of liposome synthesis. In general, liposomes with a low polydispersity and a precise control of the size were successfully produced from alteration of the flow rate ratio and channel aspect-ratio. The higher aspect-ratio enabled the most rapid generation of liposomes with similar diameter and significant lower polydispersity index than the obtained by other batch technique. Besides, β-carotene was successfully incorporated into liposomes with efficiency of approximately 60% and the incorporation ability was not specific to a choice of microfluidic device aspect-ratio. The results suggest that the use of microfluidic devices could be employed for liposome production with a possible advantage to minimize the degree of parallelization of processes. These results demonstrate the potential technical feasibility of microfluidic processes for future industrial applications.

1. Introduction

Liposomes, or phospholipid vesicles, are spherical supramolecular aggregates composed mainly by natural or synthetic phospholipids, showing shells with one or more bilayers, or lamellae, and an aqueous inner core [1,2]. Vesicle systems could be prepared using biocompatible, biodegradable, and non-toxic ingredients enabling faster and easier implementation in food systems, and overcoming regulatory barriers that might hamper their application [3]. These structures present the ability to incorporate water-soluble, lipid-soluble and amphiphilic bioactive compounds, which makes them attractive to lipid-based carrier systems for food industry [4]. The phospholipid vesicle systems have been used for delivery and/or protection of compounds such as curcumin [5], resveratrol [3], lutein [6,7], lycopene [7] and β-carotene [8–10]. Liposome application in complex food matrices has also been performed aiming to bring bench assays closer to technological scenario of food industry, such as the use of ascorbic acid-loaded liposomes in orange juice [11]; iron(III) sulfate-loaded liposomes in milk [12] and β-carotene-incorporated liposome in yogurts [13].

On the other hand, the production of phospholipid vesicles aiming food applications has several drawbacks. Among other critical factors, conventional batch techniques of liposome formation, such as film hydration, reverse-phase evaporation or alcohol injection present limitations in terms of suitability for scaling up from the bench-scale to the industrial production, since size distribution of generated liposomes show a low reproducibility from batch-to-batch [14]. Apart that, these processes are discontinuous and typically should be combined with post-processing steps to reduce the diameter and polydispersity of the final liposomes. Thus, the great challenge is the continuous production of monodisperse liposomes, with tunable sizes below the limits achieved current batch production techniques. In this context, the microfluidic techniques may be used to overcome the challenges imposed for large-scale food industrial production, due to some advantages related to specific characteristics of transport phenomena and processes scaling up.

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http://dx.doi.org/10.1016/j.colsurfb.2017.05.033
0927-7765/

Microfluidics is defined as the science of designing, manufacturing and operating processes and devices with small amounts of fluids in laminar regime. Microfluidic devices have dimensions ranging from a few millimeters to micrometers, which are characterized by exhibiting at least one channel with dimension smaller than 1 mm [15,16]. The most common microfluidic approach for liposomes production is the hydrodynamic flow-focusing technique using planar microfluidic devices with cross-shaped geometry, described firstly by Jahn et al. [17]. The microfluidic hydrodynamic flow-focusing is a simple technique for nanoscale liposome formation that provides several advantages compared with the conventional manufacturing methods [18]. In this approach, the liposome formation occurs by introducing a stream containing phospholipids dispersed in alcohol through a central channel and subsequent constriction of phospholipid dispersion by two perpendicular or oblique aqueous streams [1,17,19,20]. The controlled co-diffusion of phospholipids, alcohol and aqueous streams results in the self-assembly of unilamellar lipid nanovesicles, with diameters that may be adjusted by changing the ratio of water to alcohol volumetric flow rates [18].

Microfluidics technology offers additional advantages over batch techniques, including the possibility of liposome continuous production and scaling up by microchannels parallelization [2]. Despite of these advantages, the hydrodynamic flow-focusing technique using planar microfluidic devices for liposome formation is characterized as a low-throughput process due to the limited volumetric flow rates imposed by the small channel dimensions generally used. Scarce alternatives have been proposed to improve liposomes throughput by hydrodynamic flow-focusing, such as the increase of phospholipids concentration or total volumetric flow rate [19–21], the use of microfluidic devices with a double hydrodynamic flow-focusing region [1] or with vertical flow-focusing device [18]. The maximum flow rate and phospholipids concentration are generally restricted by limitations associated to fluid rheological behavior, which can increase internal pressure leading to clogging issues. Alternatively, the use of multiple microfluidic flow-focusing elements operating in parallel can be explored for liposomes formation. However, it is desirable to minimize the degree of parallelization, because a large number of parallel microfluidic elements can have a negative impact on the overall reliability of the continuous-flow system [18].

Thus, as an alternative to enhance the throughput of liposome synthesis by hydrodynamic flow-focusing, this work aimed to perform a systematic study of the liposome formation using planar devices with different aspect-ratio of channels. In our study, the channel aspect-ratio was defined as width/height ratio unlike height/width ratio reported previously in the literature [18]. Thus, the aspect-ratio may support industrial production of liposomal food systems with a minimal parallelization degree using a smaller number of devices. Finally, β-carotene shows important biological activity due to its provitamin A activity, but its effective utilization as nutraceutical ingredient in aqueous formulations is hampered [10,22]. For this reason, β-carotene, a hydrophobic model molecule, was incorporated in the liposomal systems, aiming the evaluation of technical feasibility of the microfluidic hydrodynamic flow-focusing as a potential technique for industrial production of food systems.

2. Materials and methods

2.1. Materials

The liposomal systems were obtained using a food-grade and deoiled powder soybean lecithin commercially named Lipoid S45 (>45% w/w phosphatidylcholine, 10–18% w/w phosphatidylethanolamine, <4% w/w lysophosphatidylcholine and <3% w/w triglycerides) acquired from Lipoid GmbH (Ludwigshafen, Germany). Absolute ethanol chromatographic-grade (99.9% v/v) and synthetic β-carotene powder (>93% w/w) were purchased from Panreac® (Barcelona, Spain) and Sigma-Aldrich (St. Louis, USA), respectively. The rigid replication masters were obtained using SU-8 25 UV photoresist and the SU-8 developer solution (MicroChem Co., Newton, MA, USA). The microfluidic devices were prepared using the commercial mixture Sylgard 184 Silicone Elastomer kit (The Dow Corning Co., Midland, MI, USA), containing the polydimethylsiloxane pre-polymer and curing agent. Polycarbonate microfluidic connectors 1.6 mm, 1/16" (Cole-Parmer Instrument Co., Chicago, IL, USA), rigid polyethylene tubes with outer diameter 2 mm (Mark Med, Bragança Paulista, SP, Brazil), silicone adhesive glue (TekBond®, Embu das Artes, SP, Brazil) and syringe needles 0.8 × 32 mm (Nipro Medical Co., Bridgewater, NJ, USA) were used to assemble the microfluidic devices.

3. Methods

3.1. Devices fabrication

Microfluidic devices were obtained using the conventional soft lithography technique, according to the protocol described by McDonald et al. [23] with some modifications proposed by Moreira et al. [24]. Firstly, four different mask layouts with flow-focusing mechanism geometry were designed using the software AutoCAD 2012 (Autodesk Inc., San Rafael, CA, USA). These flow-focusing microfluidic devices (cross-shaped junction) were designed with different channels aspect-ratio (width/height): 1:1 (50 × 50 μm), 2:1 (100 × 50 μm), 4:1 (200 × 50 μm) and 6:1 (300 × 50 μm), according to Fig. 1 (a). Next, designs were plotted in photo-masks (Fig. 1 b) with 8000 dpi resolution by DGM Design (Curitiba, Brazil). For the production of rigid replication masters, silicon wafers were spin-coated with negative SU-8 25 photoresist at 1000 rpm during 30 s. The layouts were transferred from the photo-masks to the rigid replication masters by UV exposure during 70 s using a contact mask aligner (model MJB-3 UV300, KarlSuss, Garching, Germany). Afterward, the rigid replication masters were revealed through a washing with developer solution for SU-8 25 photoresist. The microfluidic devices were casted using a polydimethylsiloxane pre-polymer and curing agent mixture (10:1 w/w). The mixture was degassed at 6 Pa during 1 h to eliminate air bubbles, and spilled over the replication masters using an appropriate experimental holder. The polymer curing process was performed during 1 h on a hot plate at 95 °C. The devices were firstly washed with ultrapure water, then with isopropyl alcohol and finally dried with nitrogen gas. The devices were irreversibly sealed to a glass slide through oxygen plasma treatment at 16 Pa during 20 s (model PLAB SE80, Plasma Technology Ltd., Wrington, England). Finally, syringe needles were inserted and sealed with silicone adhesive glue for injection of the phases and for collection of generated liposomes. Fig. 1 (b) shows a microfluidic device after the fabrication process.

3.2. Liposome production

The microfluidic production of liposomal systems was performed by hydrodynamic flow-focusing method. Basically, an alcoholic phase containing soybean lecithin dispersed in ethanol was injected between two phases containing ultrapure water, resistivity 18.2 MΩ/cm (Direct-Q3 System, Millipore Co., Billerica, MA, USA). Phases flowed through microfluidic devices with the volumetric flow rate controlled by syringe-type pumps (model PHD 2000, Harvard Apparatus, Inc.; South Natick, MA, USA). Polyethylene tubing and connectors for microfluidic were used to link the