Colloids and Surfaces B: Biointerfaces 146 (2016) 544–549

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Uniform and stable hydrogel-filled liposome-analogous vesicles with a thin elastomer shell layer

Mintae Seo[a], Aram Byun[a], Jongwon Shim[b], Hong Sung Choi[c], Youngbok Lee[d,**], Jin Woong Kim[a,d,*]

[a] Department of Bionano Technology, Hanyang University, Ansan 15588, Republic of Korea
[b] AMOREPACIFIC Co., R&D Center, Yongin 17074, Republic of Korea
[c] Shinsegae International Co., Ltd., Seoul 06015, Republic of Korea
[d] Department of Applied Chemistry, Hanyang University, Ansan 15588, Republic of Korea

ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 23 June 2016
Accepted 27 June 2016
Available online 28 June 2016

Keywords:
Vesicle
Microfluidics
Elastomer film
Impermeability

ABSTRACT

This study introduces a new type of uniform liposome-analogous vesicle with a highly stable shell structure in which water-in-oil-in-water double emulsion drops fabricated in a capillary-based microfluidic device are used as templates. The vesicles developed in this work consist of a poly(ethylene glycol) hydrogel core surrounded by a polyurethane (PU) film between 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC) layers. Subjecting the double emulsion templates to UV irradiation leads to the formation of a PU elastomer film between the DPPC layers. The presence of a thin PU film sandwiched between the DPPC layers is confirmed by confocal laser microscopy. The thicknesses of the PU films are measured to be approximately ~4 μm. Further study reveals the incorporation of the PU film between the DPPC layers remarkably improves the shell impermeability. Our vesicle system is expected to be useful for regulating the permeation of small molecules through lipid-based vesicular films.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Liposomes, which are phospholipid bilayered vesicles, have been studied regarding encapsulation and controlled drug delivery because they can load both hydrophilic molecules in the interior aqueous phase and hydrophobic molecules within the lipid bilayer [1,2]. It has been well known that these structures can also protect encapsulated molecules from degradation and from organs' passive targeting [3–6]. Hence, they have been widely investigated as outstanding carriers for therapeutics. For example, insulin encapsulated in liposomes was observed to be protected from enzymatic attacks and immune recognition because the charged lipid bilayer blocks the permeation of biomacromolecular toxins [7]. Additionally, liposome-encapsulated potent antiviral drugs, such as SPC3 for HIV-infected patients, were protected from lymphocytes and macrophages and were thus able to retain their original activity [8]. However, despite their excellent applicability, liposomes suffer from an intrinsic structural inability [9,10]. Membrane deformation, which commonly occurs during the break-up or fusion of liposome particles, results in either sharp increases in particle size or substantial loss of entrapped materials [11,12].

To overcome this limitation, versatile solutions have been suggested. Liposomes' structural stability can generally be enhanced by altering their lipid composition. For example, the incorporation of a reinforcing compound, such as cholesterol [13,14] and nano species[15–17], increases the transition temperature of lipid layers, improving their mechanical tolerance against external stresses, including heat, osmolality, and pH changes. Furthermore, crosslinking the lipid layer imbues the lipid layer with viscoelastic properties, thereby improving their structural stability [18,19]. Recent studies have also reported that hybridisation with an amphiphilic block copolymer makes the liposome both mechanically robust and chemically versatile, which are critical characteristics for improving shell permeability [20–23]. Although these studies have thoroughly demonstrated that improving the lipid layer through physical hybridisation or chemical treatment can enhance liposomes' structural stability, some leakage of the entrapped materials still occurs after storage for long periods.

In this study, we introduce a microfluidic approach to fabricate structurally stable liposome-analogous vesicles consisting of

* Corresponding author at: Department of Bionano Technology, Hanyang University, Ansan 15588, Republic of Korea.
** Corresponding author at: Department of Applied Chemistry, Hanyang University, Ansan 15588, Republic of Korea.
E-mail addresses: yblee@hanyang.ac.kr (Y. Lee), kjwoong@hanyang.ac.kr (J.W. Kim).

http://dx.doi.org/10.1016/j.colsurfb.2016.06.052
0927-7765/© 2016 Elsevier B.V. All rights reserved.

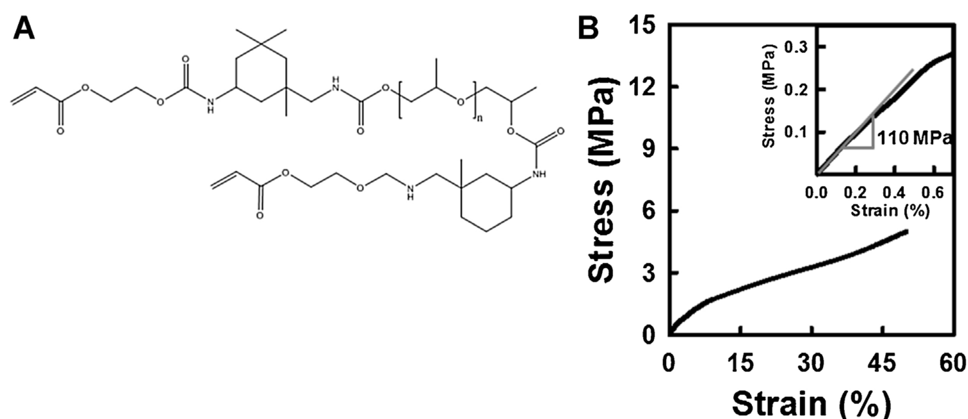

Fig. 1. (A) Molecular structure of a cross-linkable PU precursor. (B) Stress-strain curve of the PU film after UV polymerisation.

a hydrogel-filled core and a lipid bilayer that is hybridised with polyurethane (PU) elastomer. To create these vesicles, we prepare monodisperse water-in-oil-in-water (W/O/W) double emulsion templates that can be directly generated in capillary microfluidic channels. Photo-polymerisation of the core and the PU precursor in the lipid bilayer produces novel hydrogel-filled vesicles. In this vesicle system, the hydrogel immobilises the core, and the PU/lipid shell provides mechanical stability. The presence of the PU film in the lipid shell was observed to be essential in our study because it imparts viscoelastic resilience to the final vesicle (Fig. 1). Finally, we also experimentally demonstrate that our vesicle system can play an important role in regulating the permeation of small molecules through lipid films.

2. Experimental

2.1. Materials

1,2-Dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC) was supplied by Doosan Biotech (Korea). Poly(glycerin)-*b*-poly(ε-caprolactone) (PG-b-PCL) was supplied from SK Bioland Co. (Korea). Mn of PG-b-PCL was 13,500 g mol^{-1} with a polydispersity index of ~2.2. The block ratio between PG and PCL was 1.6. Polyethylene glycol-*b*-polylactic acid (PEG_{5000}-*b*-PLA_{5000}) were purchased from Gelest (USA). 1,2-Dioleoyl-*sn-glycero*-3-phosphoethanolamine (DOPE), polyvinyl alcohol (PVA, Mn = 13,000–23,000 g mol^{-1}, 87–89% hydrolysed), fluorescein sodium salt (FSS, Mn = 376.27 g mol^{-1}), poly(ethylene glycol) diacrylate (PEGDA, M_n ~6000 g mol^{-1}), sodium chloride (NaCl), and ethanol were purchased from Sigma Aldrich (USA). Texas Red-1,2-dihexadecanoyl-*sn-glycero*-3-phosphoethanolamine (Texas Red DHPE) was purchased from Invitrogen (USA). PU precursor (Secure SE-8110) was provided by Fotopolymer (Korea). 2-Hydroxy-2-methyl-1-phenyl-proan-1-one (Darocure 1173) was used as a water-soluble photo-initiator (Ciba Specialty Chemicals). Chloroform and toluene were purchased from Daejung (Korea). All other chemicals were reagent grade and used without further purification. Deionised double-distilled water was used for all experiments.

2.2. Fabrication of capillary based microfluidic devices

Our microfluidic device combined flow-focusing and co-flowing geometries [24]. A glass capillary microfluidic device was fabricated. To make a small orifice, one round capillary (outer dimeter = 1.0 mm, World Precision Instrument, USA) was tapered by heating and pulling with a pipette puller (Model P-97, Sutter Instrument, USA). The end tip of the tapered glass capillary was cut to the designated diameter using a microforge station (Micro Forge MF 830, Narishige, Japan). The small diameter was 40 μm, and the large one was 180 μm. To prevent wetting of the capillary by the aqueous fluids, the round capillaries were hydrophobically coated in toluene solution containing 1 wt% hexyltrimethoxysilane. The two tapered round capillaries were inserted into a square capillary (Atlantic International Technology, USA) with an inner diameter that was 1 mm larger than the outer diameter of the round capillary. Each end of the square capillary was fit with a needle tip and completely sealed with epoxy resin.

2.3. Generation of monodisperse W/O/W emulsion drops

The major advantage of using our capillary-based microfluidic device lies in its ability to create highly monodisperse W/O/W double emulsion drops with precisely controlled inner and outer drop sizes. As a result, the thickness of the shell can be precisely and independently tuned by simply changing the flow rate of each fluid [25,26]. In this work, the inner drop contained a 15 wt% aqueous solution of PEGDA and 0.5 wt% Darocure 1173. The middle fluid consisted of 7.5 wt% PU precursor and 0.2 wt% DPPC in a mixture of toluene and chloroform. The concentration of PU precursor was controlled from 5 wt% to 15 wt% against the mass of the middle fluid. The mixing ratio of the toluene and chloroform was 1–1.8 by volume. The outer fluid was an aqueous PVA solution (5 wt%). The three fluids were loaded into the glass syringes (Hamilton Gastight) connected to the device with polyethylene tubing (PE-5, Scientific Commodities Inc.). The flow rate of each fluid was controlled with syringe pumps (Pump 11 Elite, Harvard Apparatus), and the flow rates of the inner, middle, and outer fluids were varied in the following ranges: 100–1000 μL h^{-1}, 1000–3000 μL h^{-1}, and 5000–15,000 μL h^{-1}, respectively. The emulsion drops were collected in an aqueous solution of 90 μM NaCl to avoid osmotic stress. Finally, we were able to obtain the emulsion drops in which the O/W and W/O interfaces were stabilised with DPPC lipids.

2.4. Photo-polymerisation of hydrogel core and PU precursor

The W/O/W emulsion drops collected through the round exit capillary were solidified by photo-polymerisation. The emulsion drops were irradiated with UV light (365 nm, 500 W) for 1 min. To prevent heat generation from the UV source, the polymerisation was conducted in a cold water bath (5 °C). After polymerisation, the remaining monomers and other additives were thoroughly removed with a large amount of isopropanol by repeated centrifugation at 4000 rpm. The final samples were stored in water at room temperature.

Download English Version:

https://daneshyari.com/en/article/598781

Download Persian Version:

https://daneshyari.com/article/598781

Daneshyari.com