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Continuous preparation of bicelles using hydrodynamic focusing method for bicelle to vesicle transition

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Abstract

Bicelle is one of the most stable phospholipid assemblies, which has tremendous applications in the research areas for drug delivery or structural studies of membrane proteins owing to its bio-membrane mimicking characteristics and high thermal stability. However, the conventional preparation method for bicelle demands complicated manufacturing processes and a long time so that the continuous synthesis method of bicelle using microfluidic chip has been playing an important role to expand its feasibility. We verified the general availability of hydrodynamic focusing method with microfluidic chip for bicelle synthesis using various kinds of lipids which have a phase transition temperature ranged from -2 to 41 °C. Bicelle can be formed only when the inside temperature of microfluidic chip was over the phase transition temperature. Moreover, the concentration condition for bicelle formation varied depending on the lipids. Furthermore, the transition process characteristics from bicelle to vesicle were analyzed by effective q -value, mixing time and dilution condition. We verified that the size of transition vesicles was controlled according to the effective q -value, mixing time, and temperature.

Keywords: Bicelle, Vesicle, Dilution method, Microfluidic chip, Hydrodynamic focusing

Introduction

Lipid vesicles have critical roles in biochemical fields and nature science due to its cell membrane mimicking [1, 2]. In aqueous solution, phospholipids are self-assembled to prevent the exposure of its hydrophobic tail to aqueous buffer so that they form various types of structures, such as bilayered micelle or vesicle.

Bilayered micelle or so-called bicelle has a discoidal shape and its structure is segregated by two sections: bilayer domain section and single layer rim section which are composed of long-chain lipids and short-chain lipids, respectively. Bicelle is the most stable structure among

the phospholipid assemblies because hydrophobic area of bilayer domain is covered by short-chain lipids and stabilized [3]. Its self-stabilizing characteristics enable the retention period to be extended up to several weeks at room temperature, implying that bicelle is a suitable carrier in drug delivery systems with the incorporation of functional materials [4–6]. Furthermore, bicelle can be utilized as a pre-vesicle substance noting that it is simply transitioned to vesicle structure through dilution method [7].

Although bicelle has tremendous feasibilities in a biomedical field, its conventional preparation process, that is thin film hydration method, has a long and complicated manufacturing processes [8]. Those manufacturing processes also require harsh conditions such as freezing and thawing, repeatedly, which cause to denature the physicochemical characteristics of biological substances [9]. For these reasons, continuous manufacturing method of bicelle under mild condition has been demanded. Our

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group previously suggested the microfluidic method for bicelle synthesis without any energy exertion, as shown in Fig. 1a [10]. In this study, to verify the general availability of a hydrodynamic focusing method using microfluidic chip, the concentration and temperature condition for bicelle formation was investigated using various kinds of phospholipids, the phase transition temperature of which ranged from -2 to 41 °C. Furthermore, as shown in Fig. 1b, the prepared bicelles were transitioned to vesicles using simple dilution method with stirrer and the transition characteristics were investigated.

Experimental section

Materials

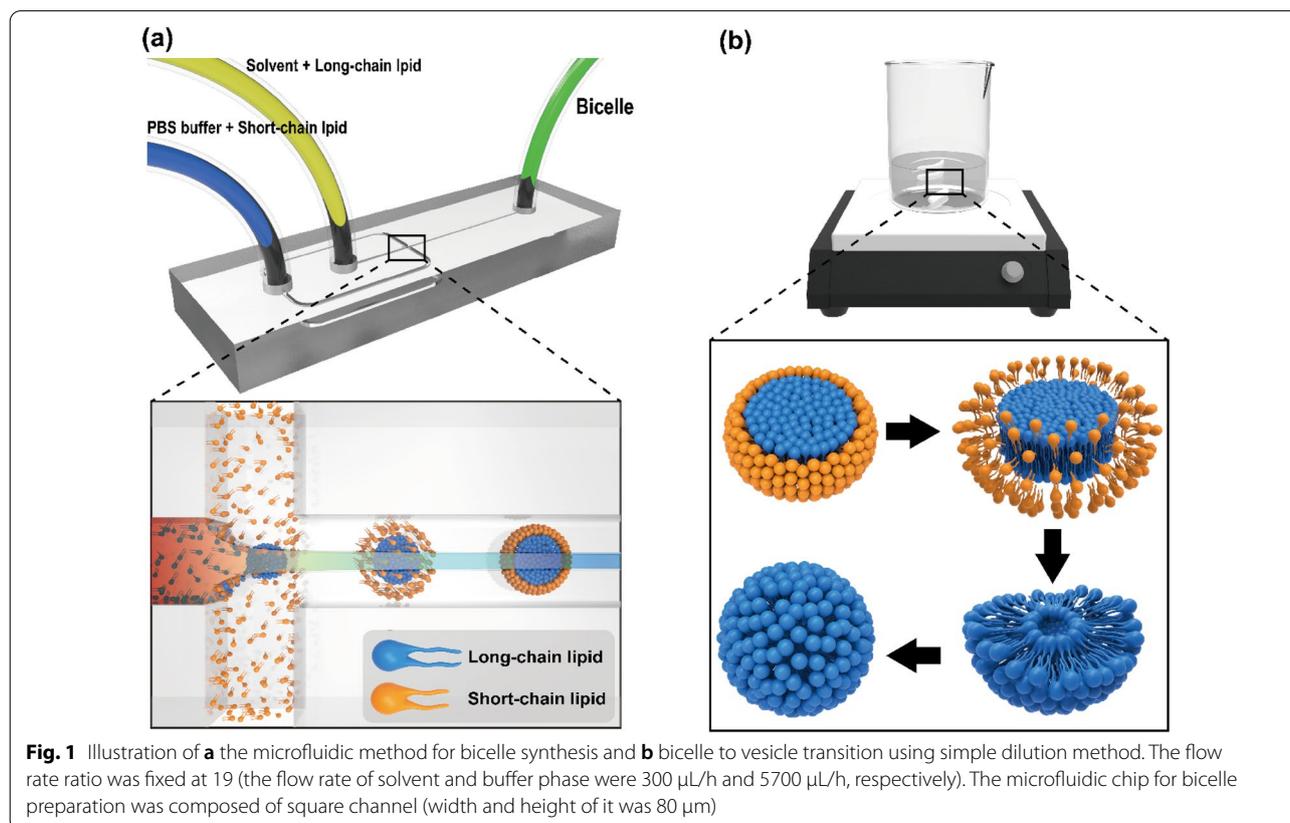
SU-8 2100 negative photoresist (PR) was purchased from MicroChem, and an SI wafer (525 μm in thickness) was used to fabricate a silicon master mold. Polydimethylsiloxane (PDMS, Sylgard184, mixed with a 10:1 ratio of curing agent) for soft molding was bought from Dow Corning; 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, $T_m = 23$ °C), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC, $T_m = -2$ °C), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, $T_m = 41$ °C) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) were procured from Avanti Polar Lipids, Inc. (Alabaster,

AL, USA) and used in preparing a DMPC/DHPC bicelle solution. Phosphate buffer saline (PBS), 1,6-diphenyl-1,3,5-hexatriene (DPH) and 6-dodecanoyl-N,N-dimethyl-2-naphthylamine (Laurdan) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used in analyzing membrane properties (fluidity and polarity). Methyl alcohol was purchased from Daejung Chemicals & Metals Co., Ltd, (Korea).

Fabrication of a microfluidic chip

A master mold was fabricated according to the manuals of negative PR and procedures of general photolithography using SI wafer. PR was uniformly coated with a thickness of 80 μm and sequentially baked at 60 °C and 90 °C. The hardened PR was treated by UV light at an intensity of 10 mJ/s with a photomask. To remove the residual PR solution, the UV-exposed SI wafer was baked and developed for 5 min [11]. Finally, the fabrication of the SI master mold for the microfluidic channel was completed.

For the fabrication of a microfluidic chip, PDMS base polymer and a curing reagent were mixed at a weight ratio of 10:1, which casted on the SI mater mold and degassed in a vacuum chamber. Degassed PDMS solution was cured at 70 °C in a convection oven for 2 h and the hardened replicate was carefully detached from the



SI master mold. PDMS microfluidic chip was sealed by binding the replicate to the planar PDMS plate using plasma treatment. The bonded chip was cured at 80 °C in a convection oven for 8 h to enhance the bonding [12].

The dimension of microfluidic chip was 11 mm × 35 mm × 8 mm. The channel was composed of cross junction with two inlets and one outlet, and the width and height of the channel was 80 μm (Aspect ratio = 1).

Preparation of lipid assemblies

PDMS microfluidic chip was used to prepare the lipid assemblies. Long-chain lipids such as DMPC, POPC, or DPPC and DHPC which are dissolved in solvent and PBS buffer (10 mM, pH 7.4) respectively, were mounted on the syringe, and injected at two different inlets. The flow rate ratio (FRR) was adjusted to 19 (Flow rate of solvent and buffer phase were 300 μL/h and 5700 μL/h). The solvent phase and the buffer phase were employed as an inner phase and outer phase, respectively, which contacted at the intersection and mixed by their mutual diffusion as shown in Fig. 1a.

The vesicles transitioned from the bicelles were prepared using the simple dilution method. Dilution was performed using 10 mM PBS buffer, equivalent to the buffer employed in preparing bicelle solution.

Size characterization by dynamic light scattering

The sizes of the lipid assemblies were measured via dynamic light scattering (DLS) measurement (Zetasizer Nano ZS90). Approximately 0.5 mL of the sample solution was loaded into a quartz cell, and scattered light intensity was measured at 20 °C (All samples were equilibrated at 20 °C for 1 min before measurement). The hydraulic radius was determined by the Stokes–Einstein equation [13, 14]:

$$r_h = \frac{k_B T}{6\pi\eta D}$$

where k_B is the Boltzmann constant, T is absolute temperature, η is the viscosity of the sample, and D is the diffusion coefficient. The refractive index and absorption values for phospholipid assemblies were 1.45 and 0.001, respectively. The viscosity and refractive index of the dispersant were 1.0143 cP and 1.3304, respectively.

Characterization of membrane properties

The membrane fluidity of lipid assemblies was evaluated with DPH [15, 16]. DPH was dissolved in a lipid solution at a molar ratio of 1:250. DPH molecules were inserted into the lipid membrane and accumulated at the middle of the bilayer. The fluorescence anisotropy of DPH was measured using a fluorescence spectrophotometer

(SCINCO, FS-2). The excitation wavelength was 360 nm, and the emission intensity was measured at 430 nm. The membrane fluidity ($1/P_{DPH}$) is defined as follows:

$$\frac{1}{P_{DPH}} = \frac{I_{\parallel} + GI_{\perp}}{I_{\parallel} - GI_{\perp}} \quad (G = i_{\perp}/i_{\parallel}),$$

where I_{\parallel} and I_{\perp} represent emission intensities parallel and perpendicular to horizontally polarized light, respectively; and i_{\parallel} and i_{\perp} represent emission intensities parallel and perpendicular to vertically polarized light, respectively.

The membrane polarity of lipid assemblies was evaluated with Laurdan [17, 18]. Laurdan was dissolved in a lipid solution at a molar ratio of 1:200. Laurdan molecules were inserted into the edge of the lipid membrane surface and accumulated on the membrane surface. The general polarization of Laurdan was measured using a fluorescence spectrophotometer (SCINCO, FS-2). The excitation wavelength was 440 nm, and the emission intensity was measured at 490 nm. The membrane polarity (GP_{340}) equation is defined as follows:

$$GP_{340} = \frac{I_{440} - I_{490}}{I_{440} + I_{490}},$$

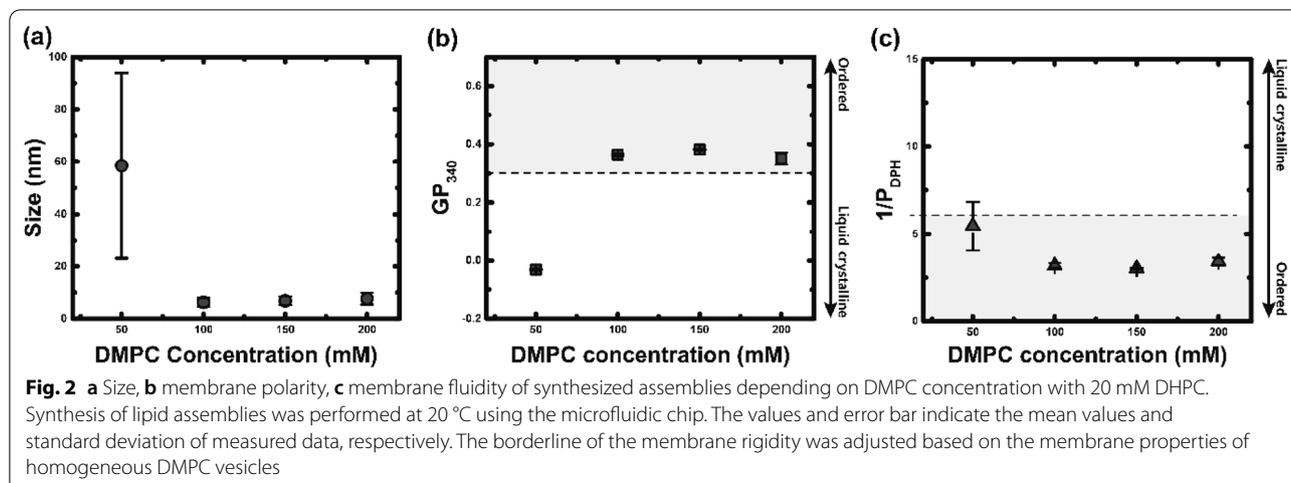
where I_{440} and I_{490} represent emission intensities of 440 and 490 nm excited with 340 nm light, respectively.

Results and discussions

Bicelle synthesis in a microfluidic system

The verification of bicelle formation was evaluated by three factors: size of assemblies, membrane properties, and morphological analysis. The small sized assemblies (<50 nm) are expected to be bicelle or mixed micelle, which can be distinguished from the membrane properties. For mixed micelle, the membrane of it comprises randomly organized two lipids so that its membrane rigidity is very low. On the other hands, bicelle is distinctly segregated by two lipids section implying that the homogeneously organized bilayer membrane of it is rigid and ordered phase. Meanwhile, relatively large sized assemblies (>50 nm) are presumably vesicles which can be classified into homogeneous and heterogeneous vesicle depending on the membrane properties. Heterogeneous membrane tends to be disordered so that the membrane rigidity is relatively low than homogeneous membrane.

The size of synthesized assemblies was investigated, first, depending on the long-chain lipid concentration, while concentration of DHPC (C6) was fixed at 20 mM. As shown in Fig. 2a, the size of the assemblies decreased sharply when DMPC (C14) concentration is

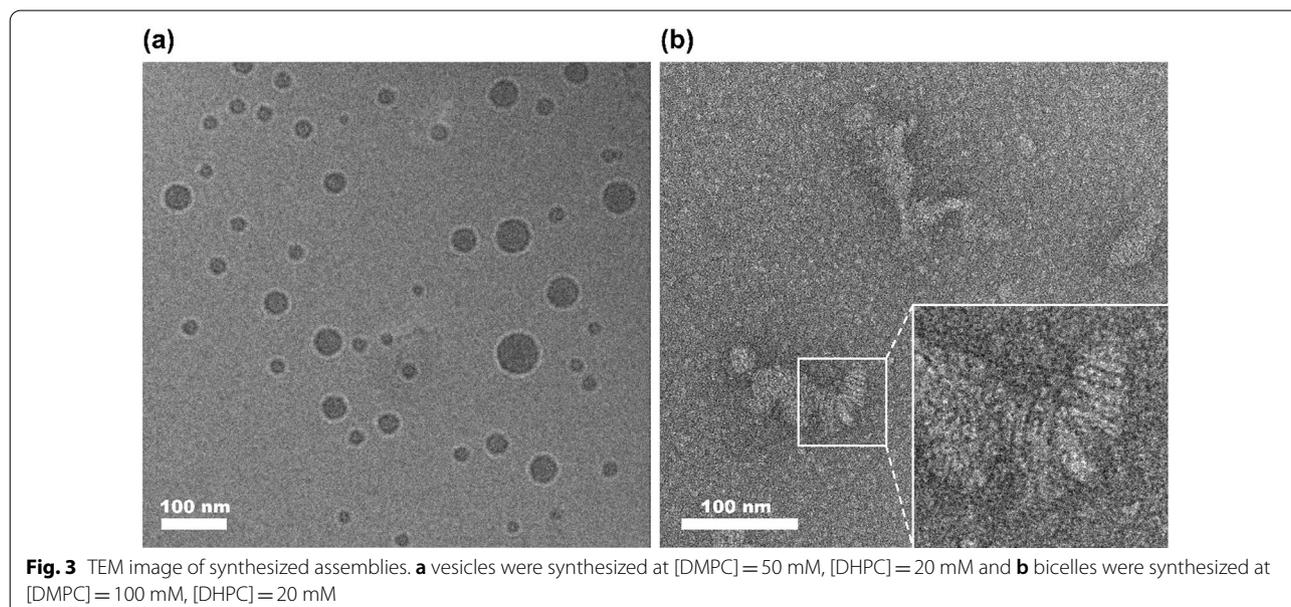


over 100 mM. The membrane polarity indicates the packing density of lipid bilayer, while the membrane fluidity refers the local viscosity of bilayer membrane. Thus, it can be noted that each property have positive and negative correlation to the membrane rigidity, respectively. For small-sized assemblies, as shown in Fig. 2b and c, DMPC membrane showed an ordered phase, implying that the bilayer membrane is homogeneous [7]. On the other hands, the relatively large assemblies have a liquid crystalline phase membrane. The membrane polarity is especially low comparison to the membrane fluidity, indicating that the orderliness of the membrane is disarranged due to the insertion of DHPC molecules, that is,

the lipid membrane of large sized assemblies is heterogeneously composed [9].

The synthesized assemblies were also analyzed via transmission electron microscope (TEM) as shown in Fig. 3. The large sized assemblies were confirmed as heterogeneous vesicles with morphological verification and membrane properties. In Fig. 3b, the assemblies were sporadically accumulated and the discoidal shape was verified, which were stacked as shown in the inset. Consequently, the bicelles were successfully prepared at high DMPC concentration condition.

Self-assembly of lipids can be described as Eq. (1) from the coalescence behavior of them. As the distance



between aggregating species decreases, the coalescence rate is accelerated. In other words, self-assembly is promoted at high lipids concentration. In this hydrodynamic focus method, it is important that DHPC molecules are supplied at an appropriate time. DHPC molecules should be supplied after an adequate bilayer domain is formed. Otherwise, the small DMPC fragments are mixed with DHPC molecules due to too slow coalescence, so that it cannot be stabilized. In such cases, the heterogeneous membrane grows longer, and it is finally vesiculated.

$$K_{ij} = \left(\frac{D_i + D_j}{\xi^2} \right) S_{ij} \xi e^{-\frac{E_{ij}}{kT}} \quad (1)$$

where D_i and D_j are diffusion coefficients of aggregating species, ξ is the distance within which coalescence can occur, $S_{ij}\xi$ is the corresponding reaction volume, E_{ij} is the fusion potential, and k is Boltzmann's constant.

Meanwhile, the size of synthesized assembly depending on POPC (C18) concentration is shown in Fig. 4a. Unlike DMPC, small-sized assemblies (~8 nm) were synthesized at low concentration. That is, POPC domains were formed with proper size before DHPC were provided, which is presumably attributed to the strong hydrophobic interaction between POPC molecules [19]. With the membrane properties in Fig. 4b and c, it is verified that the bilayer is homogeneous when POPC concentration is lower than 100 mM. Strong interaction enables the bicelle to be synthesized at low concentration, but it also induces extremely large vesicles (300–450 nm) to be formed at high concentration (> 150 mM).

Synthesis of the large vesicles due to the strong interaction is also confirmed by the experiments using DPPC (C16), as shown in Fig. 5a. The phase transition temperature of DPPC exceeds 20 °C, so that the interaction between lipids is highly enhanced. In case of DPPC, the size of the

vesicles synthesized at 20 °C were larger than POPC. On the other hands, as shown in Fig. 5b, small-sized assemblies were prepared at 45 °C, which is higher than the phase transition temperature of DPPC. For all assemblies synthesized at 45 °C, the membrane of them shows ordered gel phase, indicating that they are verified as bicelles. Consequently, it is confirmed that the concentration of the long-chain lipids plays a critical role in the microfluidic bicelle synthesis. The proper condition for bicelle formation depends on the type of the lipid, which can be controlled by considering the phase transition temperature of it.

Transition of bicelles to vesicles

DHPC molecules constitute the rim area of the bilayer domains, but also reside in buffer, monomerically. Unless the hydration extent of DHPC molecules is changed, the concentration of the monomeric DHPCs is maintained. For these reasons, dilution of the bicelle solution causes the bicellar DHPCs to be detached from rim area of bilayer domains and they become unstable owing to the exposure of hydrophobic area to buffer. The transition of bicelles to vesicles occurs when these destabilized domains coalesce and vesiculate. Here, we expected that the transition characteristics can be controlled by various factors such as physicochemical properties of bicelle or dilution condition.

The effective q -value indicates the actual composition of bicelle as a molar ratio of the long-chain lipids and short chain lipids. As the effective q -value of bicelle increases, the bilayer domain is widened so that the shape of bicelle is changed from isotropic form to planar disc. According to the shape and size of bicelle, varisized vesicles can be obtained after the dilution. As shown in Fig. 6, according to the effective q -value, the size of transitioned vesicles was entirely different.

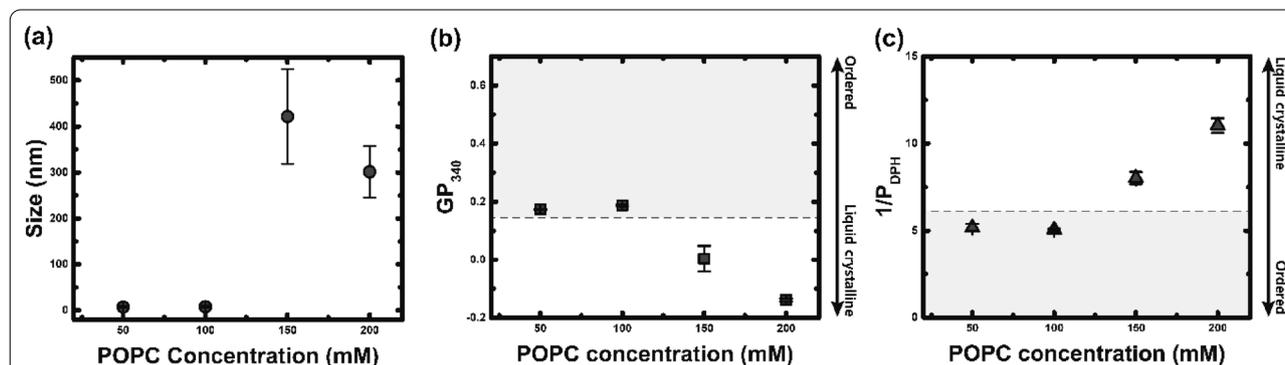
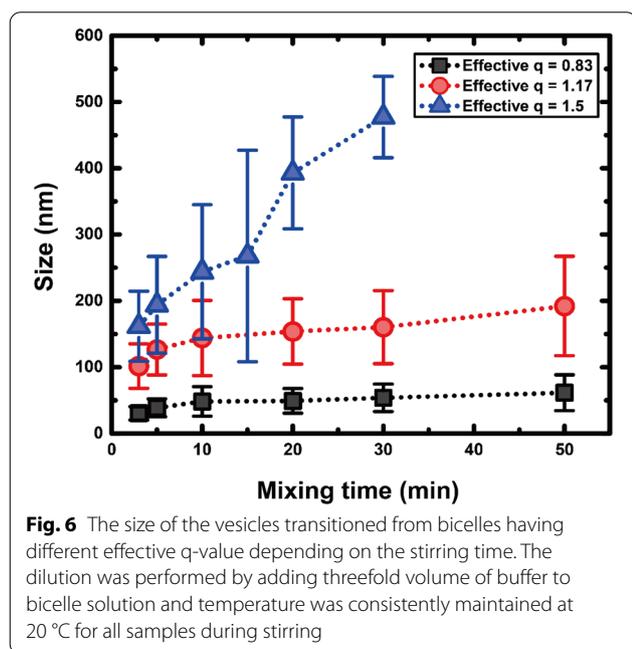
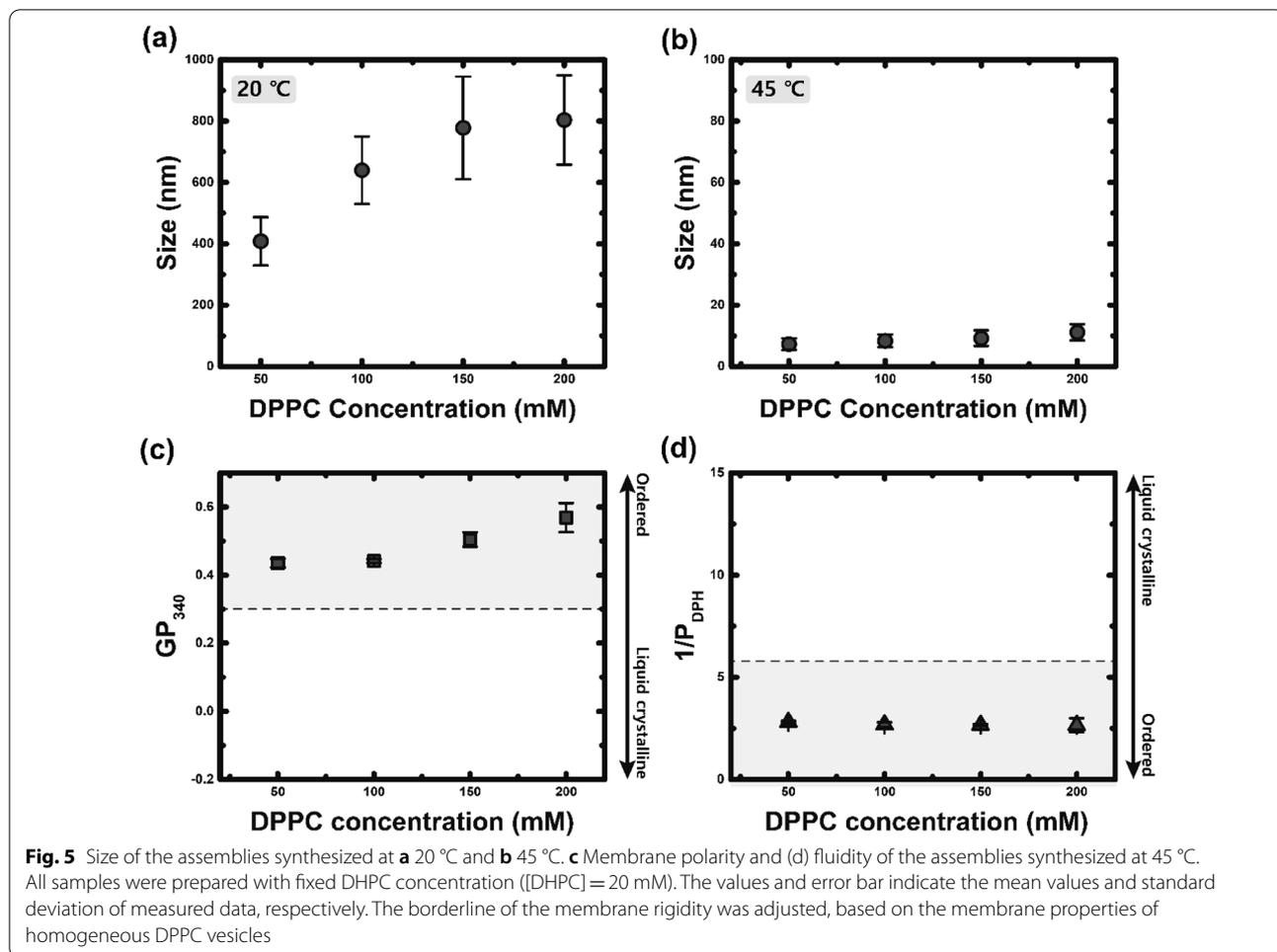


Fig. 4 a Size, b membrane polarity, c membrane fluidity of synthesized assemblies depending on POPC concentration with 20 mM DHPC. Synthesis of lipid assemblies was performed at 20 °C using the microfluidic chip. The values and error bar indicate the mean values and standard deviation of measured data, respectively. The borderline of the membrane rigidity was adjusted, based on the membrane properties of homogeneous POPC vesicles



The effective q -values were obtained from Eq. (2), and the concentration of monomeric DHPC was referred from the previous results [10]. To exclude the effect of DMPC concentration, all samples were prepared at a fixed DMPC concentration. For high effective q -value bicelle, it tends to be large-sized vesicle, which is attributed to the widened bilayer domain of it and fast detachment of DHPC molecules from its rim.

$$q^* = \frac{[DMPC]}{[DHPC]_{bicellar}} = \frac{[DMPC]}{[DHPC]_{total} - [DHPC]_{monomeric}} \tag{2}$$

where q^* indicates the effective q -value and $[DMPC]$, $[DHPC]_{bicellar}$, $[DHPC]_{total}$, and $[DHPC]_{monomeric}$ are the concentration of DMPC, bicellar DHPC, total DHPC, and monomeric DHPC, respectively.

In addition, mixing time using a magnetic stirrer also affects the size of the transitioned vesicles. With longer mixing time, more fragments could be coalesced, so that large vesicles were obtained. As a result, a wide

range of vesicles can be prepared using bicelles according to the effective q -value and mixing time.

Meanwhile, as confirmed in the synthesis of DPPC bicelle (Fig. 5), temperature plays a prominent part in determining the structure of lipids assembly. The formation of vesicles can be described as the balance between the line tension and rigidity of the bilayer domain (Eq. 3) [20]. The vesiculation index indicates that the vesiculation occurs when the line tension is large or the bending modulus is small enough to bend over and close into a sphere. The line tension of the bilayer domain not only increases, but also the bending modulus of it decreases with increasing temperature, indicating that the vesiculation is accelerated at high temperature condition [21, 22]. Thus, antedated vesiculation cause the size of the transitioned vesicles to be small, which is experimentally confirmed in Fig. 7.

$$V_f = \frac{r\Lambda}{4\kappa} = \frac{r\Lambda_0}{4\kappa} \left[1 + \frac{kT}{\alpha_b} \ln(1 - \phi_r) \right] \quad (3)$$

where r and Λ indicate the length and line tension of the bilayer domain, respectively. Λ_0 is the line tension without stabilization by DHPC, κ is the bending modulus, α_b is the energy gain from stabilization by one molecule, and ϕ_r is the fraction of the rim of the bilayer domain.

Conclusions

Bicelle is expected to play a critical role in a drug delivery system due to its high stability. The hydrodynamic focusing method for bicelle preparation is predominant in terms of a productivity and maintaining the activity of the functional materials. In this study, the

general availability of the microfluidic chip for bicelle synthesis was investigated from a point of view the critical conditions for bicelle formation was experimentally identified in the perspective of the lipids concentration and temperature. Bicelle formation was determined by investigating the physicochemical properties of the synthesized assemblies such as the size, morphological structures and membrane properties.

Indeed, the transition of bicelle to vesicle was analyzed according to the effective q -value and dilution conditions. Vesicle size is a principal factor in a drug delivery system, due to its influences on a cellular uptake, transportation, and accumulation behavior. With three types of bicelle, the size of the transitioned vesicles was investigated by controlling the mixing time. In addition, it is confirmed that the temperature dependency of the line tension and bending modulus of intermediete domains also affects the size of vesicles. Consequently, a wide range of the vesicles were obtained from the bicelles and it presumably contributes to broadening the feasibility of the lipid assemblies. In this study, the stirring speed of the magnetic stirrer was fixed and the transition characteristics were investigated in terms of temperature and stirring time. However, the stirring speed is considered as an important parameter because the shear-induced vesicle fusion could be accelerated with the increased stirring speed. To verify the effect of the stirring speed, the experiments should be reorganized and we hope that it can be addressed in further studies.

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Authors' contributions

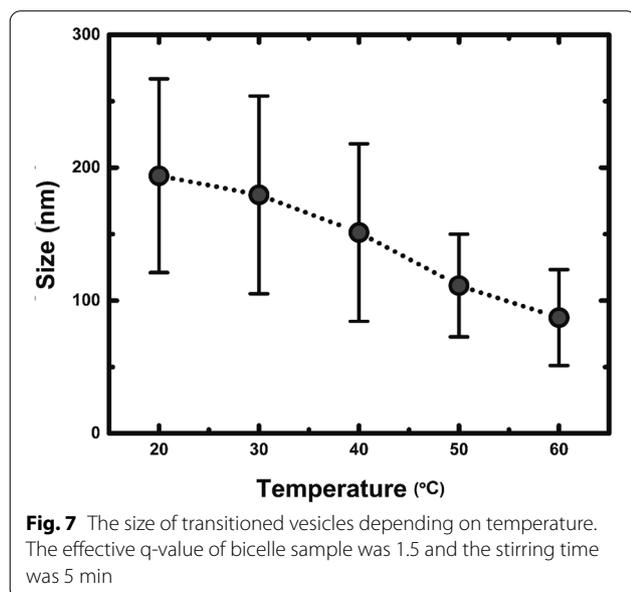
SHC and BSK designed the study. SH.C. and BS.K. collected the data, analyzed and interpreted the results, and drafted the manuscript. HSJ and TS and KSK analyzed and interpreted the data. KSK. and HSJ. conceived the study and assisted in the analyses. KSK and TS and HSJ conceived the study, interpreted the data, funding acquisition and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published.



Declarations

Competing interests

The authors declare that they have no competing interests.

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References

- Mhashal AR, Roy S (2015) Self-assembly of phospholipids on flat supports. *Phys Chem Chem Phys* 17:31152–31160. <https://doi.org/10.1039/C5CP03358F>
- Yang S, Jiang L (2020) Biomimetic self-assembly of subcellular structures. *Chem Commun* 56:8342–8354. <https://doi.org/10.1039/D0CC01395A>
- Sanders CR, Prosser RS (1998) Bicelles: a model membrane system for all seasons? *Structure* 6:1227–1234. [https://doi.org/10.1016/S0969-2126\(98\)00123-3](https://doi.org/10.1016/S0969-2126(98)00123-3)
- Hirva S, Jenisha P (2016) Bicelle: a lipid nanostructure for transdermal delivery
- Lin L, Wang X, Guo Y et al (2016) Hybrid bicelles as a pH-sensitive nano-carrier for hydrophobic drug delivery. *RSC Adv* 6:79811–79821. <https://doi.org/10.1039/C6RA18112K>
- Wu H, Su K, Guan X et al (2010) Assessing the size, stability, and utility of isotropically tumbling bicelle systems for structural biology. *Biochimica et Biophysica Acta (BBA)* 1798:482–488. <https://doi.org/10.1016/J.BBAMEM.2009.11.004>
- Taguchi S, Kang BS, Suga K et al (2020) A novel method of vesicle preparation by simple dilution of bicelle solution. *Biochem Eng J* 162:107725. <https://doi.org/10.1016/J.BEJ.2020.107725>
- Hameed S, Bhattarai P, Dai Z (2018) Cerasomes and Bicelles: Hybrid Bilayered Nanostructures With Silica-Like Surface in Cancer Theranostics. *Front Chem* 6:127. <https://doi.org/10.3389/fchem.2018.00127>
- Taguchi S, Suga K, Hayashi K et al (2018) Systematic characterization of DMPC/DHPC self-assemblies and their phase behaviors in aqueous solution. *Colloids Interfaces*. <https://doi.org/10.3390/colloids2040073>
- Choi SH, Kang BS et al (2021) A simple method for continuous synthesis of bicelles in microfluidic systems. *Langmuir*. <https://doi.org/10.1021/acs.langmuir.1c02024>
- Mata A, Fleischman AJ, Roy S (2006) Fabrication of multi-layer SU-8 micro-structures. *J Micromech Microeng* 16:276–284. <https://doi.org/10.1088/0960-1317/16/2/012>
- Kim P, Kwon K, Park M, et al (2008) Soft lithography for microfluidics
- Hassan PA, Rana S, Verma G (2015) Making sense of Brownian motion: colloid characterization by dynamic light scattering. *Langmuir* 31:3–12. <https://doi.org/10.1021/la501789z>
- Yamamoto T, Tayakout-fayolle M, Iimura K et al (2019) Effect of high pressure on growth of colloidal particles during sol–gel phase transition of resorcinol–formaldehyde solution. *Adsorption*. <https://doi.org/10.1007/s10450-019-00042-4>
- Fox MH, Delohery TM (1987) Membrane fluidity measured by fluorescence polarization using an EPICS V cell sorter. *Cytometry* 8:20–25. <https://doi.org/10.1002/cyto.990080104>
- van Dam L, Karlsson G, Edwards K (2004) Direct observation and characterization of DMPC/DHPC aggregates under conditions relevant for biological solution NMR. *Biochimica et Biophysica Acta (BBA)* 1664:241–256. <https://doi.org/10.1016/J.BBAMEM.2004.06.005>
- Haverstick DM, Glaser M, Gratton E (1987) Visualization of Ca²⁺-induced phospholipid domains. *Proc Natl Acad Sci* 84:4475–4479. <https://doi.org/10.1073/pnas.84.13.4475>
- Parasassi T, Krasnowska EK, Bagatolli L, Gratton E (1998) Laurdan and Prodan as Polarity-Sensitive Fluorescent Membrane Probes
- Heerklotz H, Blume A (2012) 5.5 Detergent interactions with lipid bilayers and membrane proteins. *Compreh Biophys* 5:63–91. <https://doi.org/10.1016/B978-0-12-374920-8.00529-4>
- Zook JM, Vreeland WN (2010) Effects of temperature, acyl chain length, and flow-rate ratio on liposome formation and size in a microfluidic hydrodynamic focusing device. *Soft Matter* 6:1352–1360. <https://doi.org/10.1039/B923299K>
- Méléard P, Gerbeaud C, Pott T et al (1997) Bending elasticities of model membranes: influences of temperature and sterol content. *Biophys J* 72:2616–2629. [https://doi.org/10.1016/S0006-3495\(97\)78905-7](https://doi.org/10.1016/S0006-3495(97)78905-7)
- Yi Z, Nagao M, Bossev DP (2009) Bending elasticity of saturated and monounsaturated phospholipid membranes studied by the neutron spin echo technique. *J Phys*. <https://doi.org/10.1088/0953-8984/21/15/155104>

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