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Microfluidic platforms with monolithically integrated hierarchical apertures for the facile and rapid formation of cargo-carrying vesicles

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Hyesung Cho,[abc1] Junsoo Kim,[d1] Keishi Suga,[e1] Takaaki Ishigami,[e] Hyunchul Park,[f] Jung Won Bang,[bc] Soonmin Seo,[g] Mansoo Choi,[abc] Pahn-Shick Chang,[h] Hiroshi Umakoshi,*[e] Ho-Sup Jung*[a] and Kahp-Yang Suh[i]

We fabricated a simple yet robust microfluidic platform with monolithically integrated hierarchical apertures. This platform showed efficient diffusive mixing of the introduced lipids through approximately 8,000 divisions with tiny pores (~5 μm in diameter), resulting in a massive, real-time production of various cargo-carrying particles via a multi-hydrodynamic focusing.

Synthetic particles carrying therapeutic compounds have been rationally designed with cell-like membranes consisting of bilayers of amphiphilic lipids, to enhance their cargo-carrying ability for use in a wide range of biomedical applications, such as delivery of vaccines and drugs.[1-6] In order to prepare a liposome, which is a cargo-carrying particle consisting of a spherical, unilamellar lipid membrane, procedures utilizing the multiple extrusion of multilamellar vesicles through a polycarbonate membrane have been developed.[7] However, the widespread utilization of such methods has been restricted in practical areas owing, in part, to the fact that it takes several hours to form liposomes through the lipid film formation, freeze-thawing process, and repeat extrusion process. Hence, alternative microfluidic methods have been developed for the rapid and direct formation of various types of vesicles by designing special microfluidic techniques or platforms,[8,9] including jetting via micro-nozzle,[10,11] sheath flow[12-15] or microvortices[15] within microchannels. The advantage of nozzle-based jetting techniques lies in the uniform size distribution of the obtained vesicles because every droplet is independently generated through the same nozzle, whose diameter is fixed throughout the process; however, such a serial jetting technique is typically limited in throughput due to its discontinuous flow control over time.

By contrast, diffusion-based methods utilizing a continuous sheath flow or microvortices in specific microfluidic channels have been developed to yield high quantities of lipid vesicles by inducing the passive/active diffusive mixing of lipids at the water/oil interfaces within the platforms. These techniques work because the lipid assemblies in oil solutions undergo specific structural changes to form spherical vesicles spontaneously when the concentration of the lipid solution is rapidly changed.[13,17] In this regard, the rapid hydrodynamic focusing of the lipid solution within the water phase leads to a number of diffusion processes, resulting in the rapid formation of vesicles in an attempt to maintain energy balance at the mixing region where the concentration of the lipids is rapidly dropped. Despite their simplicity, mixing based techniques typically require a tiny platform with a narrow channel width to ensure uniform and efficient mixing conditions at the focused water/oil interface,[13] which potentially prevents the real-time, massive production of particles. Moreover, the facile size control of particles, proper drug loading ability, and batch-to-batch reproducibility should be further improved to accelerate the practical use of these vesicles in upcoming biomedical applications.[1]

To address the above challenges, we report herein a simple yet robust microfluidic platform for the facile and rapid formation of cargo-carrying particles by reliably embedding a polymeric membrane with monolithically integrated hierarchical apertures that function as an array of multi-nozzles. The key idea is that an array of hierarchical apertures with ordered tiny pores within the microfluidic platform induces highly uniform and controlled lipid diffusion against the internal laminar flows by continuously generating a number of multi-hydrodynamic focal points via individual nozzles. In our platform, the efficient diffusion and mixing of the introduced lipids occurs through 8,000 tiny nozzles (~5 μm in diameter), which rationally combines both the advantages of single-nozzle-based jetting and diffusion-based mixing methods and can be used as a guide for the highly uniform and real-time production of lipid vesicles in a facile manner.

Microfluidic platform with hierarchical apertures

The schematic illustration in Fig. 1a shows the basic definition of the hierarchical apertures, which exploits a concept of vertical attachment with polymeric layers having different size pores. Because a typical thin polymeric membrane (< 10 μm in thickness) is prone to be fragile due to its reduced mechanical strength, it is potentially difficult to maintain the shape of thin apertures without tears or cracks against normal pressure. However, the presented concept produced robust membrane systems with pores by hierarchical levelling that two layers with different size apertures are fabricated in one membrane layer to

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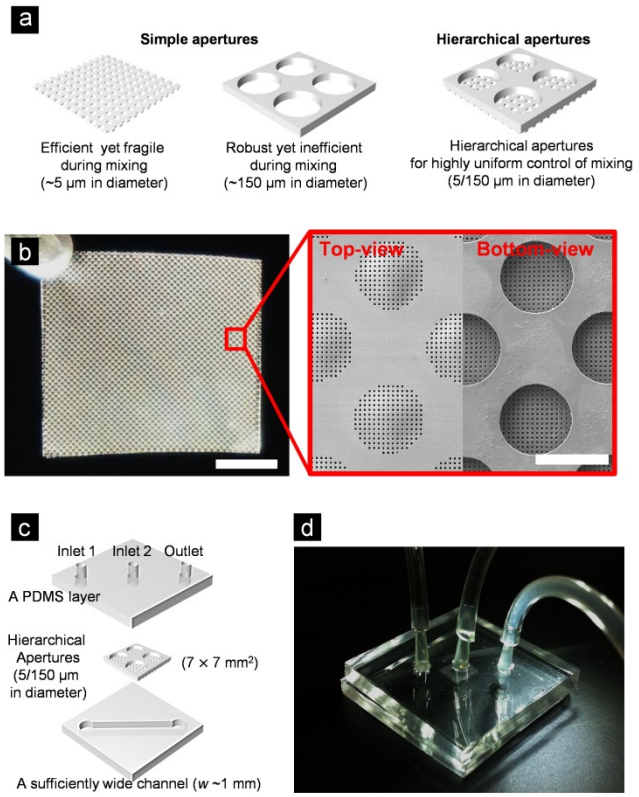

Fig. 1 A schematic illustration for the hierarchical aperture-embedded microfluidic platform. (a) Conceptual comparison in apertures for a robust and highly efficient control of mixing. The larger pores support the membrane to protect the fragile and weak, smaller pores by providing geometrical reinforcement. (b) Microscopic and SEM images showing the robust two-level hierarchical apertures. (c) Illustration of the construction of the microfluidic platform by incorporating each polymeric layer, where a polymeric membrane with ordered hierarchical apertures as a multi-nozzle-layer is shown. (d) Digital image of the constructed PDMS channel with an array of hierarchical apertures. Scale bars in (b) and inset of (b) are represent 5 mm and 150 μm, respectively.

obviously elevate the mechanical strength. Recently, we reported that the hierarchical mould could rapidly expel the excessive resin within a sandwich-like moulding setup via a soft-lithography process, which leads a robust generation of hierarchical apertures with various polymers.[18] For this study, a droplet of hydrophilic resin, Norland Optical Adhesive (NOA73, USA), was drop-dispensed onto a hydrophobic, two-level hierarchical mould having 5-μm pillars on an array of 150-μm dots. Then, a flat hydrophobic blanket was placed over the mould to dewet the NOA resin. After performing a dewetting process in a short period of time (< 2 min), the set-up was fully exposed to ultraviolet (UV) light (< 3 min, λ=250-400 nm) under a slight pressure (< 100 g/cm^2). When the top flat blanket was peeled off, a binary dewetting surface without a residual layer of NOA was produced. Finally, a polymeric membrane with monolithically integrated hierarchical apertures was obtained by carefully removing the bottom hierarchal mould from the assembly. The obtained polymeric membranes with an array of ordered two-level hierarchical apertures are shown in the Fig. 1b, where defect-free and free-standing polymeric (NOA73) membranes with designed tiny apertures are neatly formed over a large area (1.5 × 1.5 cm^2). The membrane has a large number of robust 5-μm nozzles on top of the layer, as observed from the top-view (inset in Fig. 1b), while the same 5-μm pores are also observed through the 150-μm apertures via the bottom-view, because it was directly replicated from the mould with two-level hierarchical patterns.

A similar geometrical reinforcement of seemingly fragile apertures by hierarchical levelling also occurs in the human body.[19] The glomerular basement membranes in kidney systems play an important role as bio-filters that permit only small plasmas to pass into the urinary space via a thin membrane with small pores, where the membrane is robustly supported by a type of podocyte layer (or pedicel layer) to enhance the mechanical strength in order to withstand the high blood pressure. The structural similarity between the two-level hierarchical apertures in this study and the glomerular basement membrane system suggests that the apertures could be reliably layered into a multi-nozzle system for successful embedding in microfluidic platforms. The schematic illustration in Fig. 1c depicts three major parts to complete the hierarchical aperture-embedded microfluidic platform. Two polydimethylsiloxane (PDMS, Dowcorning, Korea)) layers were replicated from a pre-patterned Si master; one layer had three holes, for two inlets and one outlet (the hole diameter of the syringe tube connection was 1 mm), and the other layer had a wide channel (width: 1 mm, depth: 100 μm, length: 2 cm), where the membrane with hierarchical apertures was directly connected to the inlet 2. To prevent leakage around the membrane apertures, an uncured NOA73 coating was applied to the bottom channel. Note that the channel surface was not coated with resin during this step to allow the PDMS channel to maintain its original dimensions. After assembling the membrane within the PDMS layers, a slight UV exposure was performed to cure the set-up. The digital image in Fig. 1d shows the fabricated hierarchical aperture-embedded microfluidic platform.

Multi-hydrodynamic focusing for a facile formation of vesicles

The membrane has a number of two-level apertures; one layer has an array of ordered 5-μm apertures (3 × 10^5/cm^2) to finely divide the introduced liquid sample and to yield multi-hydrodynamic focusing via each pore, and the other layer has 150-μm apertures to provide geometrical reinforcement for the tiny pores. Note that our platform rapidly reduces the concentration of introduced isopropyl alcohol (IPA, Kanto Chemical Co., Inc. (Tokyo, Japan)) by aperture-enhanced diffusion via multi-hydrodynamic focusing because the lipid solution passes through approximately 8,000 divisions of tiny apertures in the platform (Fig. 2a). When an IPA solution with lipids (10,12-pentacosadiynoic acid, PCDA (Sigma Aldrich, US), or 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine, DMPC (Avanti Polar Lipid, Inc. (Alabaster AL, U.S.A.))) was continuously introduced into inlet 2 and passed through the individual 5-μm pores, multi-hydrodynamic focusing was made around each pore because both the underlying shear flow of water from inlet 1 and the jet flow through the tiny aperture were laminar flows with a low Reynolds number (Re << 1). In general, a laminar flow within the microfluidic channel is known to prevent the convective mixing of liquids. However, our platform, using many individual apertures, yielded 8,000 separated and individual microflows, inducing the rapid and proficient formation of uniform vesicles in an attempt to maintain energy balance by enabling the effective diffusion of molecules between IPA and water. To form lipid vesicles via presented platform, a lipid solution in IPA (10 mM) was introduced to inlet 2 using a syringe pump, while deionized water was introduced to inlet 1. The total flow rate was controlled at 1 ml/min. By changing the flow rate, lipid vesicles were collected from the outlet according to various FRR

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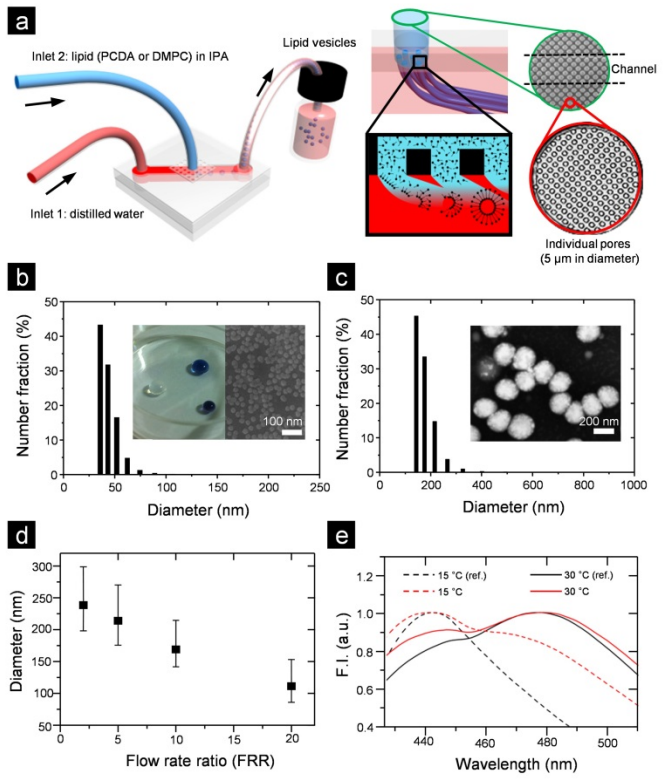

Fig. 2 A rapid and facile formation of cargo-carrying particles using presented microfluidic platform with an array of hierarchical apertures. (a) Multi-hydrodynamic focusing is induced in the platform, which enhances the diffusive mixing of the lipid molecules and results in the direct formation of spherical lipid vesicles to maintain energy balance. (b) Dynamic light scattering (DLS) results of the PCDA vesicles, showing the uniform size distribution of the obtained vesicles. The inset in (b) is a digital image to show colour response after a photo-crosslinking an SEM image of the formed PCDA vesicles. (c) DLS results and transmission electron microscopy (TEM) images show the skewed narrow size distribution of the obtained DMPC vesicles at a constant FRR of 10. (d) Straightforward size modulation of the lipid vesicles is possible by selecting different FRRs. (e) Phase transition of the obtained DMPC vesicles (red) and conventional DMPC liposomes (black) at different temperatures.

values (2, 5, 10, and 20). The final sample of lipid vesicles was heated at 50 °C for 20 min to remove the IPA from the solution. The hydrodynamic size of the lipid vesicle was measured using a particle analyzer (ELSZ-1000, Photal Otsuka Electronics, Japan).

PCDA, a type of photo-polymerizable lipid, was selected to visualize the shape and size distribution of the formed particles after polymerization via UV light to form ene-yne alternating polymer chains (Fig. 2b). As shown in the inset, obtained sample showed no significant colour, while blue-colour response was monitored after the photo-polymerization. Compared with bulk mixing without using a microfluidic platform,[20] our method produces a more highly uniform size distribution of the formed vesicles, as measured by dynamic light scattering (DLS; ELSZ-1000, Otsuka electronics, Japan). As shown in the inset, the hierarchical aperture-embedded platform yields highly uniform and mono-disperse PCDA vesicles (diameter: 45.0 ± 9.5 nm). In addition to the photo-polymerizable lipid, DMPC, a type of phospholipid that is a component of cell membranes was also used to form the cell-like lipid vesicles (Fig. 2c). It should be noted that the sum of the two main size fractions of the formed lipid vesicles exceeded 75% when the flow rate ratio (FRR, flow rate of water/flow rate of IPA) was 10, which indicates good size uniformity. Moreover, the size of the formed lipid vesicles could be modulated in a facile manner by changing the FRR values because the mixing between the introduced water and the IPA solution could be controlled by regulating the speed of the underlying water flow near the pores (Fig. 2d, mean diameter = 111.3, 169.6, 214.0, and 238.6 nm for FRRs of 20, 10, 5, and 2, respectively). Because the vesicle diameter was small (100-200 nm), it is assumed that the prepared vesicle can be unilamellar. In this case, all other conditions except for the FRR were fixed, such as the total flow rate (1 ml/min) and the concentration of the DMPC solution (10 mM).

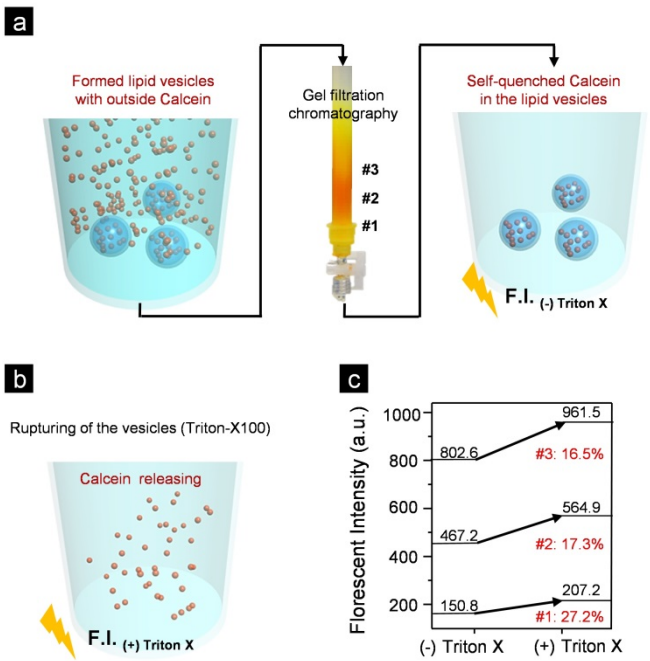

Fig. 3 Evaluation of the encapsulation efficiency of the lipid vesicles. (a) Illustration of gel-filtration to remove unincorporated calcein molecules. (b)Triton X-100 treatment to rupture the formed vesicles. (c) An evaluation of the encapsulation efficiency of the lipid vesicles with 10 mM calcein according to the fraction over time.

The physicochemical properties of the obtained vesicles were also investigated by comparing membrane polarity of obtained vesicles with that of lipid vesicles from a conventional membrane-extraction method. A fluorescent probe, 6-Lauroyl-2-dimethylamino naphthalene (Laurdan, Wako Pure Chemicals (Osaka, Japan)), was used to monitor the phase transition of the vesicles (Fig. 2e). The Laurdan peaks at 440 nm and 480 nm indicated a solid-ordered phase (gel) and a liquid-disordered phase (fluid), respectively.[21,22] The Laurdan spectra were monitored by fluorospectroscopy (FP-6500 and FP-8500; Jasco, Tokyo, Japan) at different sample temperatures (15 and 30 °C). For comparison with the conventional method, DMPC liposomes with a mean diameter of 100 nm were prepared by Bangham's method. In the case of the DMPC liposome obtained from the conventional membrane-extrusion method, the main Laurdan peak

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was observed at approximately 440 nm when the temperature of the vesicle sample was 15 °C (black dashed line), whereas heating (30 °C) above the phase transition temperature (T_c = 23 °C) shifted the peak to 480 nm (black line).[23] Similarly, the lipid vesicles produced by our platforms showed the same tendency at the same temperature (red lines in Figure 3e), suggesting that the vesicles have similar membrane properties as those of conventional DMPC liposomes although they were formed in the microchannel directly.

Evaluation of the cargo-carrying ability of obtained vesicles

To further probe the encapsulation efficiency (e.e.: defined as the concentration of molecules inside the vesicle per that contained in the loaded solution), calcein (Sigma-Aldrich (St. Louis, MO, USA)) was chosen as a water-soluble drug model.[24] After the preparation of vesicles with 10 mM calcein (pH = 7.5), their fluorescence intensities were analyzed (Ex = 490 nm and Em = 525 nm). Here, the e.e. was calculated as follows:

$$\text{e.e.} = (I_{(+)\text{Triton X}} - I_{(-)\text{ Triton X}}) / I_{(+)\text{Triton X}} \quad (1)$$

where $I_{(+)\text{Triton X}}$ and $I_{(-)\text{ Triton X}}$ are the fluorescence intensities of calcein after and before treatment with Triton X-100, respectively, and, ($I_{(+)\text{Triton X}} - I_{(-)\text{ Triton X}}$) and ($I_{(+)\text{Triton X}}$) represent the calcein concentration inside the vesicle and that in the loaded solution, respectively (Fig. 3ab). A lipid suspension in IPA solution (lipid: 10 mM) was mixed with distilled water containing 10 mM calcein (pH = 7.5) using the microfluidic platform. The prepared vesicle suspension was heated at 50 °C for 20 min and then kept in the dark at 4 °C. The external calcein molecules were removed from the vesicle fraction by gel filtration chromatography with a Sepharose 4B column (diameter: 1 cm, length: 15 cm, Sepharose 4B was purchased from GE Healthcare (Little Chalfont, UK)). The column was washed with an excess of 10 mM NaCl (Wako Pure Chemicals (Osaka, Japan)). Then, 1 ml of vesicle suspension was applied to the column at room temperature, and the vesicle fraction was collected over time. The fluorescence intensities of calcein in the vesicle samples were analyzed (Ex = 490 nm and Em = 525 nm), where each sample was diluted 100-fold with a 10 mM NaCl solution. Then, the vesicle samples were treated at 50 °C for 30 min with Triton X-100 (1 wt.%), which is a nonionic surfactant that disrupts vesicle bilayers. After the treatment, the fluorescence intensity of calcein was measured. After the vesicle sample was separated using gel filtration chromatography and collected in each vial (~2 ml), where the faster fraction of the sample showed significant intensity changes. The Fig. 3c shows the intensity changes before and after the treatment. Because the calcein is self-quenched at the high concentration used (> 10 μM), the intensity increased upon the release of calcein induced by rupturing the vesicle by Triton X-100 treatment. Previous reports have determined that the e.e. values of calcein in DMPC vesicles[25] and in DMPC-betamethasone vesicles[26] were ca. 20 % and 14.5-24.1 %, respectively. In our results, the e.e. of the DMPC vesicles formed with microfluidic platform was approximately 16.5-27.2%, although varying from the fraction in the column, which is an adequate value for drug delivery applications that do not require specific lipid handling techniques, such as lipid film drying, hydration, freezing-thawing, and extrusion procedures.

Conclusions

In this paper, we demonstrated a facile route for the massive formation of mono-disperse lipid vesicles based on microfluidic platforms with monolithically integrated hierarchical apertures in robust polymeric membranes. The obtained vesicles showed good membrane properties and encapsulation efficiencies and could effectively deliver the cargo substance, although they were directly and massively formed within a microfluidic channel with a large channel width (~1 mm). The presented platform rationally combined the advantages of both nozzle-based jetting and diffusion-based mixing methods, and multi-hydrodynamic focusing was easily achieved via the nozzle-like, multi-level apertures. The structural reinforcement of the tiny apertures was also possible by monolithically integrating robust 150-μm apertures to give hierarchical levelling to the seemingly fragile tiny apertures (5 μm in diameter). The presented platform circumvents the need for time-consuming processes in the formation of vesicles, and the device is sufficiently robust; therefore, this platform provides a simple, economically viable, and facile route for the preparation of uniform lipid vesicles suitable for drug delivery applications, which could accelerate the practical use of drug-carrying particles.

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Notes and references

[a] Department of Mechanical and Aerospace Engineering, Seoul National University, Seoul, Republic of Korea, E-mail: jhs@snu.ac.kr
[b] Global Frontier Center for Multiscale Energy Systems, Seoul National University, Seoul 151-744, Republic of Korea
[c] Division of WCU Multiscale Mechanical Design, Department of Mechanical and Aerospace Engineering, Seoul National University, Seoul, Republic of Korea
[d] Energy Harvesting Devices Research Section, Electronics and Telecommunications Research Institute, Daejeon 305-700, Republic of Korea
[e] Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, Osaka, Japan, E-mail: Hiroshi.Umakoshi @cheng.es.osaka-u.ac.jp
[f] Center for Materials Architecturing, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea
[g] College of BioNano Technology, Gachon University, Gyeonggi, Republic of Korea
[h] Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea
[i] Deceased
[1] These three authors contributed equally to this work
* Corresponding author

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1. P. M. Valencia, O. C. Farokhzad, R. Karnik and R. Langer, *Nat. Nanotechnol.*, 2012, **7**, 623-629.
2. M. J. Sailor and J. H. Park, *Adv. Mater.*, 2012, **24**, 3779-3802.
3. J. J. Moon, H. Suh, A. Bershteyn, M. T. Stephan, H. P. Liu, B. Huang, M. Sohail, S. Luo, S. H. Um, H. Khant, J. T. Goodwin, J. Ramos, W. Chiu and D. J. Irvine, *Nat. Mater.*, 2011, **10**, 243-251.
4. D. Peer, J. M. Karp, S. Hong, O. C. FaroKHzad, R. Margalit and R. Langer, *Nat. Nanotechnol.*, 2007, **2**, 751-760.
5. M. N. Holme, I. A. Fedotenko, D. Abegg, J. Althaus, L. Babel, F. Favarger, R. Reiter, R. Tanasescu, P. L. Zaffalon, A. Ziegler, B. Muller, T. Saxer and A. Zumbuehl, *Nat. Nanotechnol.*, 2012, **7**, 536-543.
6. J. J. Shi, A. R. Votruba, O. C. Farokhzad and R. Langer, *Nano Lett.*, 2010, **10**, 3223-3230.
7. F. Olson, C. A. Hunt, F. C. Szoka, W. J. Vail and D. Papahadjopoulos, *Biochim. Biophys. Acta. Biomembr.*, 1979, **557**, 9-23.
8. D. van Swaay and A. deMello, *Lab Chip*, 2013, **13**, 752-767.
9. R. R. Hood, D. L. DeVoe, J. Atencia, W. N. Vreeland and D. M. Omiatek, *Lab Chip*, 2014, **14**, 2403–2409.
10. J. C. Stachowiak, D. L. Richmond, T. H. Li, A. P. Liu, S. H. Parekh and D. A. Fletcher, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 4697-4702.
11. A. Seth, G. Bealle, E. Santanach-Carreras, A. Abou-Hassan and C. Menager, *Adv. Mater.*, 2012, **24**, 3544-3548.
12. A. Jahn, W. N. Vreeland, M. Gaitan and L. E. Locascio, *J. Am. Chem. Soc.*, 2004, **126**, 2674-2675.
13. A. Jahn, S. M. Stavis, J. S. Hong, W. N. Vreeland, D. L. Devoe and M. Gaitan, *Acs Nano*, 2010, **4**, 2077-2087.
14. M. Rhee, P. M. Valencia, M. I. Rodriguez, R. Langer, O. C. Farokhzad and R. Karnik, *Adv. Mater.*, 2011, **23**, H79-H83.
15. P. M. Valencia, P. A. Basto, L. F. Zhang, M. Rhee, R. Langer, O. C. Farokhzad and R. Karnik, *Acs Nano*, 2010, **4**, 1671-1679.
16. Y. T. Kim, B. L. Chung, M. M. Ma, W. J. M. Mulder, Z. A. Fayad, O. C. Farokhzad and R. Langer, *Nano Lett.*, 2012, **12**, 3587-3591.
17. M. Antonietti and S. Forster, *Adv. Mater.*, 2003, **15**, 1323-1333.
18. H. Cho, J. Kim, H. Park, J. Won Bang, M. Seop Hyun, Y. Bae, L. Ha, D. Yoon Kim, S. Min Kang, T. Jung Park, S. Seo, M. Choi and K. Y. Suh, *Nat. Commun.*, 2014, **5**, 3137.
19. W. G. Bae, H. N. Kim, D. Kim, S. H. Park, H. E. Jeong and K. Y. Suh, *Adv. Mater.*, 2014, **26**, 675-700.
20. A. Jahn, J. E. Reiner, W. N. Vreeland, D. L. DeVoe, L. E. Locascio, M. Gaitan, *J. Nanopart. Res.*, 2008, **10**, 925-934.
21. K. Suga and H. Umakoshi, *Langmuir*, 2013, **29**, 4830-4838.
22. T. Parasassi, G. De Stasio, G. Ravagnan, R. M. Rusch and E. Gratton, *Biophys. J.*, 1991, **60**, 179-189.
23. J. M. Boggs, *Biochim. Biophys. Acta. Rev. Biomembr.*, 1987, **906**, 353-404.
24. T. Shimanouchi, H. Ishii, N. Yoshimoto, H. Umakoshi and R. Kuboi, *Colloids Surf., B*, 2009, **73**, 156-160.
25. A. Gillet, A. Grammenos, P. Compere, B. Evrard and G. Piel, *Int. J. Pharm.*, 2009, **380**, 174-180.
26. L. Trevino, F. Frézard, M. Postel, and J. G. Riess, *J. Liposome Res.*, 1994, **4**, 1017-1028.

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