Macromolecules

Volume 40, Number 13 June 26, 2007

Communications to the Editor

Perforated Block Copolymer Vesicles with a Highly Folded Membrane

Yongming Chen,* Jianzhong Du,[†] Ming Xiong, and Hongxia Guo*

State Key Laboratory of Polymer Physics and Chemistry,
Joint Laboratory of Polymer Science and Materials,
Institute of Chemistry, The Chinese Academy of Sciences,
Beijing 100080, P. R. China

Hiroshi Jinnai* and Takeshi Kaneko

Department of Macromolecular Science and Engineering,
Graduate School of Science and Engineering,
Kyoto Institute of Technology, Goshokaido-cho,
Matsugasaki, Kyoto 606-8585, Japan

Received February 6, 2007
Revised Manuscript Received May 14, 2007

Closure of block copolymer bilayer in the solution generates the so-called polymeric vesicles or polymersomes with the solvent fills in their internal spaces. Recently, polymeric vesicles have attracted a great attention not only from academia but also from application field for drug delivery.[1] Simple block copolymer vesicles, spherical topology with one compartment, have been well recognized. Furthermore, the complex vesicles like onions,[2] compound vesicles,[3] and tube-walled vesicles[4] have been observed experimentally. For the vesicles from lipids, limited perforated vesicles with one and two pores, toroidal topology, have been reported.[5] Though deformation and perforation of lipid vesicles have been studied for some time,[6] the perforated vesicles from block copolymers have been reported scarcely. Recently, several groups have reported the perforation of block copolymer membrane by using simulation.[7] Experimental observation of such an unusual structure and exploration of how it formed are very important for understanding the vesicle evolution. However, to the best of our knowledge, the highly perforated polymeric vesicle of polybutadiene-*block*-poly(ethylene oxide) (PB-*b*-PEO) is the only one example reported so far, and this report failed to unveil the fine structure of vesicles due to the limitation of optical microscopy.[8]

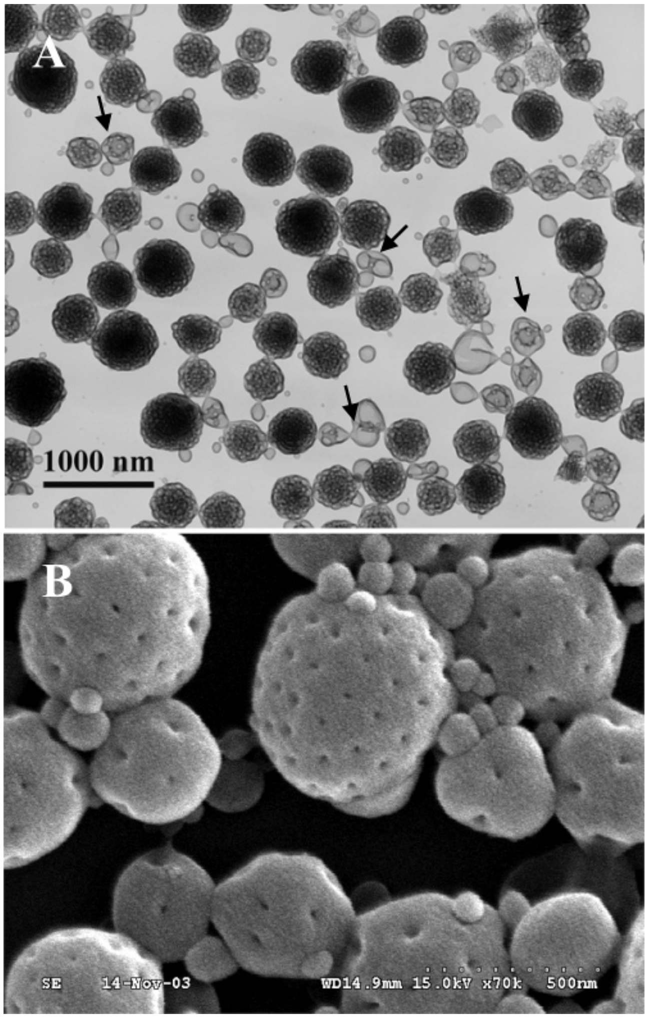

Figure 1. (A) TEM (the arrows point to the deformed vesicles with holes) and (B) SEM images of the gelated vesicles of PEO_{45}-*b*-$PTMSPMA_{180}$ in a mixture of methanol and water.

---

* To whom correspondence should be addressed. E-mail: ymchen@iccas.ac.cn and hjinnai@kit.ac.jp.

[†] Present address: Department of Chemistry, The University of Sheffield, UK.

10.1021/ma0703195 CCC: $37.00

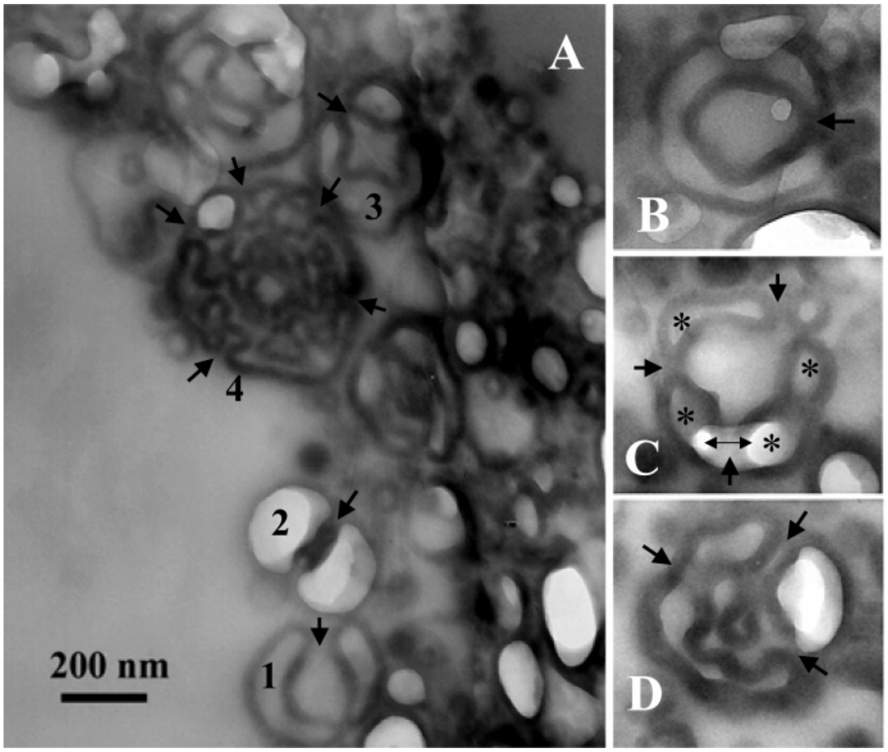

Figure 2. TEM images of (A) the microtomed slice of complex vesicles and (B−D) the selected slices from different places. The arrows point to the passages; the asterisks in (C) indicate the continuous areas, and the double-head arrow in (C) points to an internal channel.

In this paper, we report the observation of perforated vesicles with internal structure from the diblock copolymer, poly(ethylene oxide)-*block*-poly(3-(trimethoxysilyl)propyl methacrylate) (PEO-*b*-PTMSPMA), by using electron microscopy analysis together with transmission electron microtomography (TEMT). In contrast with the reported polymeric vesicles of PB-*b*-PEO, there are highly folded bilayers, except the perforated surface in the present observation. Previously, we have demonstrated that PEO_{45}-*b*-$PTMSPMA_{59}$ forms simple vesicles in the mixture of methanol and water.[9] As triethylamine was introduced, the gelation process occurred in the PTMSPMA layer of vesicle wall, and as a result, the vesicles transformed into cross-linked organic/inorganic hybrid hollow particles. Furthermore, we found that this block copolymer in mixture of *N,N′*-dimethylformamide and water generated compound vesicles, whose cross section was a honeycomb structure, possibly due to the collision and fusion of simple vesicles.[10] However, the present block copolymer, PEO_{45}-*b*-$PTMSPMA_{180}$, with a longer PTMSPMA block formed the new complex vesicles with a highly folded membrane.

The aggregation of PEO_{45}-*b*-$PTMSPMA_{180}$ was induced by adding water into the polymer solution of methanol, 1 mg/mL, until water content reached 39 wt %. After stirring for 5 h, triethylamine, 0.4 wt %, was added to trigger the gelation reaction to fix the morphology [Note: the ungelated particles were too soft to be stable without solvent]. Figure 1A displays TEM image of the particles after gelation process. Since the silica atoms scattered stronger relatively, the observed dark lines were regarded as the silica-rich membranes, whereas the PEO corona was not visible. All the particles observed have a hollow structure with a uniform wall of ca. 20 nm, which corresponds to a bilayer structure owing to the self-assembling of block copolymer. A few hollow particles are simple vesicles, but the majority is far from simple. As pointed by the arrows in Figure 1A, the particles seem to have holes or passages inside. From Figure S1 (see Supporting Information) of the same sample, we may also observe some vesicles with only one hole, which are so-called one handle vesicles.[5] At a first glance, these hollow particles with a patterned internal structure looks like the compound vesicles we observed previously.[3,10] However, as presented in the SEM analysis shown in Figure 1B, there are small holes on the surface, which is completely different from the smooth surface of the compound vesicles we observed previously.

In order to identify how the holes were arranged inside the vesicles, the samples were embedded into epoxy resin and microtomed. Figure 2 gives the TEM image of one slice and some selected images from different slices, from which the particles with internal structure can be found. The passages, pointed by arrows, were observed clearly. As indicated by the arrows, both particle 1 and 2 in Figure 2A had a passage crossover, indicating they are vesicles with one passage. Particle 3 in Figure 2A is another vesicle with two passages (at least in this slice of vesicle). The particle in Figure 2B is an inward curved vesicle. Figure 2C,D displays the vesicles with more holes (should have more for whole particles). These holes interconnected inside the vesicles. In present slice, the continuous holes seem to divide the whole particle into several parts, as indicated by the asterisks in Figure 2C. But these parts are actually interconnected with each other in three dimensions. It was noteworthy that the transparent place, indicated by a double-head arrow in Figure 2C, was an area that was not filled by the epoxy resin during embedding, and it seems a channel connecting two neighboring spaces.

Particle 4 in Figure 2A, corresponding to those big and complex vesicles presented in Figure 1, has an intricate structure of highly folded membranes. However, these bilayers are intertwined, and it is hard to understand their three-dimensional structure. Therefore, we have applied a transmission electron microtomography (TEMT) to analyze one particle by collecting

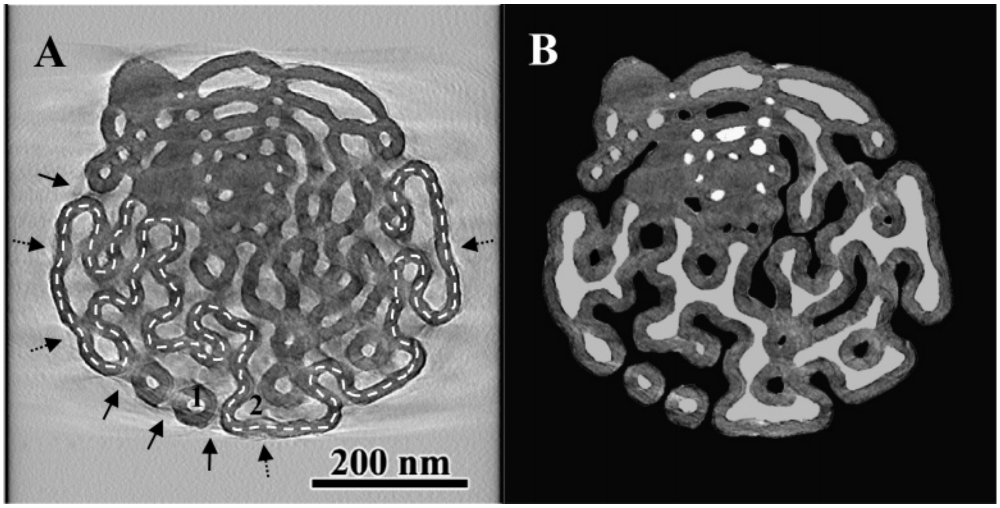

Figure 3. (A) A digital slice with pores pointed by arrows, and a dotted line is used to help the sight line to follow one continuous bilayer. The solid arrows point to the pores in the present slice; the dotted arrows show those pores not in this slice but located in the neighboring slices which are found with the aid of video 1. (B) Artwork reproduced from image A. The black areas are continuous in the space and connected with the outside through the passages, whereas the gray areas are also continuous but divided by a bilayer.

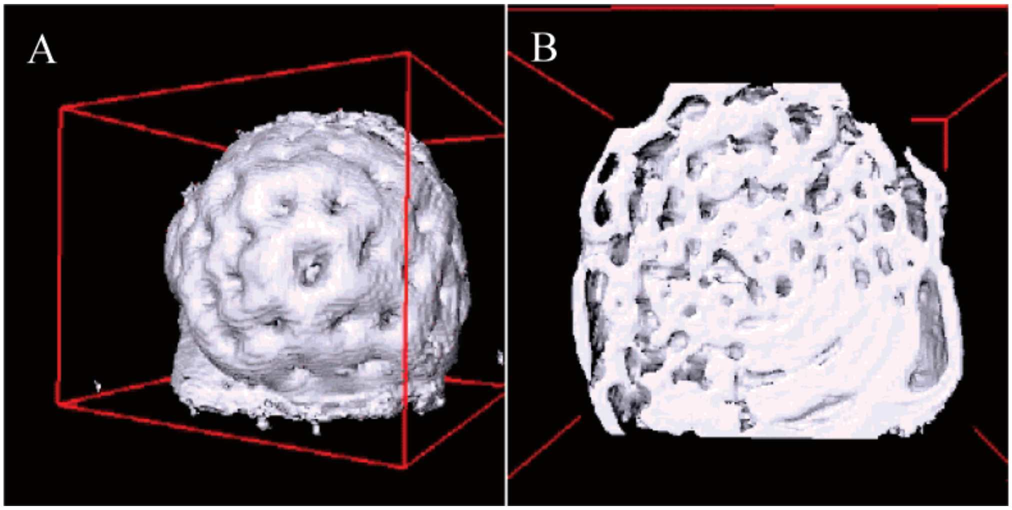

Figure 4. A surface (A) and cross-section 3D image (B) of the particle in Figure 3. Box size is 630 × 630 × 400 nm.

a series of tilted transmitted images in two directions. After making a reconstruction, the three-dimensional structure became available (see detailed method and video 1 in the Supporting Information).[11] An orthogonal cross-sectional view of 3D reconstruction for one complex vesicle is displayed in Figure S2 (Supporting Information). The Z axis is parallel to the depth direction. The $X-Y$ plane is the view of a digital slice from top of the particles, whereas the $X-Z$ and $Y-Z$ planes are the side view of slices from different direction. Obviously, the whole particle is composed of an interconnected membrane that is so flexible like a fluid membrane (note the gelation had frozen the structure of self-assembly).

Figure 3A shows one slice image in order to make a detailed description. As indicated by the solid arrows, there are at least 10 passages with a diameter of 15−25 nm. Note around the places pointed to by the dashed arrows there are more holes located in the other slices with the aid of video 1 (see snapshots in Figure S3 in the Supporting Information). The reconstructed 3D morphology shown in Figure 4A (and video 2 in the Supporting Information) provides a further proof of the perforated structure. In order to display the structure clearly, the image of Figure 3A was reproduced with the aid of the videos. As shown in Figure 3B, those areas which were actually continuous in 3D were filled with same color. Thus, some “isolated” areas, such as 1 and 2 in Figure 3A, are actually continuous. Furthermore, it is clear that the two areas in one particle, black and gray, are bicontinuous in the real space divided by a bilayer. The cross section in 3D images shown in Figure 4B also supported this conclusion. All these measurements indicate that the big particle is a perforated vesicle with a highly folded internal bilayer.

In present system, though the basic bilayer structure is the same, the morphology looks diverse: simple vesicles, low and high passage vesicles with inner structure coexisted, indicative of a nonequilibrium state. The coexisting behavior in block copolymer self-assembly has been explained by several factors.[7b,12−14] What is most fascinating is the perforated surface and highly folded inner membrane obtained herein. For the perforated vesicles by PB-*b*-PEO block copolymer, minimization of the elastic energy of the membrane under the constraints of fixed volume, membrane area, and spontaneous curvature is identified as the underlying mechanism of the observed local equilibrium shape.[8] These constraints induce the fluidic membrane to change its shape continuously and randomly to form various shape and exhibit conformal diffusion in perforated lipid vesicles.[5d] Also, the stalk-pore mechanism of membrane fusion may be involved in perforation, in which the anisotropic stalk expansion can lead to stalk-bending with one or two pores in one or each of the two fusing membrane as explored in several computer simulations.[7] We therefore speculate that the formation of perforated block copolymer vesicles with internal membrane may be due to either bending one lamella in several places or by aggregation of individual vesicles followed by a fusion process or by a combination of both under a kinetic control. Because of the longer hydrophobic chain length of PTMSPMA, the slow kinetics associated with the high molecular masses may greatly hinder structural evolution toward a global equilibrium, the intermediates are relatively long-lived, and the bilayers are perforated with the trapped pores. After perforation the passages are formed by the solvent molecules dissolved in pores. These passages are interconnected and form a single phase, which is separated from the isolated space by the bilayer membrane. The entire structure is bicontinuous, resembled a sponge with holes or channels through which solvent molecules can move, and is probably a trapped metastable structure rather than an equilibrium one.

In conclusion, block copolymer PEO_{45}-*b*-$\mathrm{PTMSPMA}_{180}$ in solution is found to form into novel perforated vesicles. There are holes on vesicle surface which are interconnected inside, and there are isolated spaces inside as well. These holes and spaces, separated by a highly folded bilayer membrane, are continuous in 3D. Experimental observation of such unusual structure can be very important for understanding the evolution of block copolymer vesicles and the biomembrane deformation.

Acknowledgment. Y.C. thanks financial support from NSF China (50473056, 20534010, and 20625412). H.J. is grateful to NEDO for support through the Japanese National Project “Nano Structured Polymer Project” by the Ministry of Economy, Trade and Industry, Japan. This work is partially supported by the Ministry of Education, Science, Sports and Culture, Japan (18550194 and 19031016).

Supporting Information Available: Experimental section, Figure S1, Figure S2, Figure S3, video 1, and video 2. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Some recent review articles: (a) Antonietti, M.; Förster, S. *Adv. Mater.* **2003**, *15*, 1323. (b) Kita-Tokarczyk, K.; Grumelard, J.; Haefele, T.; Meier, W. *Polymer* **2005**, *46*, 3540.

(2) Harris, J. K.; Rose, G. D.; Bruening, M. L. *Langmuir* **2002**, *18*, 5337.

(3) Zhang, L.; Eisenberg, A. *Macromolecules* **1996**, *29*, 8805.

(4) Yu, K.; Bartels, C.; Eisenberg, A. *Macromolecules* **1998**, *31*, 9399.

(5) (a) Seifert, U. *Phys. Rev. Lett.* **1991**, *66*, 2404. (b) Fourcade, B.; Mutz, M.; Bensimon, D. *Phys. Rev. Lett.* **1992**, *68*, 2551. (c) Jülicher, F.; Seifert, U.; Lipowsky, R. *Phys. Rev. Lett.* **1993**, *71*, 452. (d) Michalet, X.; Bensimon, D. *Science* **1995**, *269*, 666.

(6) Luisi, P. L., Walde, P., Eds. *Giant Vesicles*; John Wiley & Sons: New York, 2000.

(7) Hole formation by simulation: (a) Müller, M.; Katsov, K.; Schick, M. *J. Polym. Sci., Part B: Polym. Phys.* **2003**, *41*, 1441. (b) Sevink, G. J.; Zvelindovsky, A. V. *Macromolecules* **2005**, *38*, 7502. (c) Srinivas, G.; Discher, D. E.; Klein, M. L. *Nano Lett.* **2005**, *5*, 2343. (d) Noguchi, H.; Takasu, M. *J. Chem. Phys.* **2001**, *115*, 9547. (e) Noguchi, H.; Takasu, M. *Phys. Rev. E* **2001**, *64*, 41913. (f) Marrink, S. J.; Mark, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 11144.

(8) Haluska, C. K.; Góźdź, W. T.; Döbereiner, H. G.; Förster, S.; Gompper, G. *Phys. Rev. Lett.* **2002**, *89*, 238302.

(9) Du, J. Z.; Chen, Y. M.; Zhang, Y. H.; Han, C. C.; Fischer, K.; Schmidt, M. *J. Am. Chem. Soc.* **2003**, *125*, 14710.

(10) Du, J. Z.; Chen, Y. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 5084.

(11) (a) Jinnai, H.; Nishikawa, Y.; Ikehara, T.; Nishi, T. *Adv. Polym. Sci.* **2004**, *70*, 115. (b) Sugimori, H.; Nishi, T.; Jinnai, H. *Macromolecules* **2005**, *38*, 10226.

(12) Zhang, L. F.; Eisenberg, A. *Polym. Adv. Technol.* **1998**, *9*, 677.

(13) Zhu, J.; Jiang, Y.; Liang, H. J.; Jiang, W. *J. Phys. Chem. B* **2005**, *109*, 8619.

(14) Won, Y. Y.; Brannan, A. K.; Davis, H. T.; Bates, F. S. *J. Phys. Chem. B* **2002**, *106*, 3354.

MA0703195