Dimension Control of Glycolipid Nanotubes by Successive Use of Vesicle Extrusion and Porous Template

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We have developed a rapid and convenient method to prepare lipid nanotubes (LNTs) with defined diameters. Extrusion of the aqueous dispersion of a glycolipid *N*-(11-*cis*-octadecenoyl)-*β*-D-glucopyranosylamine at 90 °C through a polycarbonate (PC) filter (100 nm pore size) produced uniform vesicles with thin walls. The extruded vesicles were filled into the nanopores of an anodic alumina membrane (AAM) filter (200 nm pore size) and then self-assembled into tubular structures within the nanopores on cooling. This method enabled us to narrow the distribution of outer diameters, inner diameters, and wall thickness of the resultant LNTs. The mean outer diameter of the LNTs was 148 nm, approaching the pore size of the PC filter used for the extrusion. The extrusion through the 100 nm PC filter effectively reduced the mean wall thickness of the LNTs to 48 nm.

Introduction

With the development of nanotechnology, nanostructures of diverse shapes and dimensions, such as spheres, rods, fibers, and tubules, have emerged for microelectronic and biological applications. Among the nanostructures, the tubular structures with nanometer-scaled cylindrical hollows have attracted much attention in terms of possible applications based on their unique shapes.[1−6] Nanotubes made of various inorganic, organic, or hybrid materials, including carbon,[7] metal,[8,9] metal oxide,[10] peptides,[11] lipids,[12−14] and polymers,[15,16] have been prepared to provide desired functions. Lipid nanotubes (LNTs) with hydrophilic surfaces, composed of biocompatible lipid molecules, have potential use in biology and medicine.[14,17−20] Furthermore, appropriate chemical modification of the inner surfaces of the LNTs allows us to provide novel nanotube hosts for selective encapsulation of mesoscale guest substances.[21] Although the self-assembled LNTs have many advantageous characteristics, the self-assembly process also offers limited flexibility with respect to the dimensions of nanotubes, as pointed out by Steinhart et al.[22] Multiwall LNTs consisting of the glycolipid used in this study generally exhibit broad distribution of outer diameters ranging from a few hundred nanometers to a few micrometers. Several thermodynamic methods allowed us to prepare LNTs with uniform diameters.[17] These methods are, however, time-consuming and LNTs of definite dimensions are still unavailable. On the other hand, in-pore self-assembly of lipids using polycarbonate (PC) membranes as templates could produce nanotubes with defined outer diameters.[23] However, preparation of LNTs with defined inner diameters and wall thickness is still difficult even if one can employ this template method. In the present paper, we describe a simple and effective method to prepare LNTs with defined dimensions including outer diameters, inner diameters, and wall thickness. Using a conventional extrusion

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(1) Martin, C. R.; Kohli, P. *Nat. Rev. Drug Discovery* **2003**, *2*, 29.
(2) Kohli, P.; Martin, C. R. *Curr. Pharm. Biotechnol.* **2005**, *6*, 35.
(3) Kohli, P.; Martin, C. R. *J. Drug Delivery Sci. Technol.* **2005**, *15*, 49.
(4) Shim, M.; Kam, N. W. S.; Chen, R. J.; Li, Y. M.; Dai, H. J. *Nano Lett.* **2002**, *2*, 285.
(5) Mitchell, D. T.; Lee, S. B.; Trofin, L.; Li, N. C.; Nevanen, T. K.; Soderlund, H.; Martin, C. R. *J. Am. Chem. Soc.* **2002**, *124*, 11864.
(6) Wong, S. S.; Joselevich, E.; Woolley, A. T.; Cheung, C. L.; Lieber, C. M. *Nature* **1998**, *394*, 52.
(7) Iijima, S. *Nature* **1991**, *354*, 56.
(8) Martin, C. R. *Adv. Mater.* **1991**, *3*, 457.
(9) Martin, C. R. *Science* **1994**, *266*, 1961.
(10) Lakshmi, B. B.; Dorhout, P. K.; Martin, C. R. *Chem. Mater.* **1997**, *9*, 857.
(11) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324.
(12) Schnur, J. M. *Science* **1993**, *262*, 1669.
(13) Evans, E.; Bowman, H.; Leung, A.; Needham, D.; Tirrell, D. *Science* **1996**, *273*, 933.
(14) Shimizu, T.; Masuda, M.; Minamikawa, H. *Chem. Rev.* **2005**, *105*, 1401.
(15) Parthasarathy, R. V.; Martin, C. R. *Nature* **1994**, *369*, 298.
(16) Steinhart, M.; Wendorff, J. H.; Greiner, A.; Wehrspohn, R. B.; Nielsch, K.; Schilling, J.; Choi, J.; Gosele, U. *Science* **2002**, *296*, 1997.
(17) Kamiya, S.; Minamikawa, H.; Jung, J. H.; Yang, B.; Masuda, M.; Shimizu, T. *Langmuir* **2005**, *21*, 743.
(18) Yui, H.; Shimizu, Y.; Kamiya, S.; Yamashita, I.; Masuda, M.; Ito, K.; Shimizu, T. *Chem. Lett.* **2005**, *34*, 232.
(19) Banerjee, I. A.; Yu, L. T.; Matsui, H. *Nano Lett.* **2003**, *3*, 283.
(20) Matsui, H.; Porrata, P.; Douberly, G. E. *Nano Lett.* **2001**, *1*, 461.
(21) Kameta, N.; Minamikawa, H.; Goutev, N. V.; Rim, J. A.; Jung, J. H.; Shimizu, T. *Adv. Mater.* **2005**, *17*, 2732.
(22) Steinhart, M.; Wehrspohn, R. B.; Gosele, U.; Wendorff, J. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1334.
(23) Porrata, P.; Goun, E.; Matsui, H. *Chem. Mater.* **2002**, *14*, 4378.

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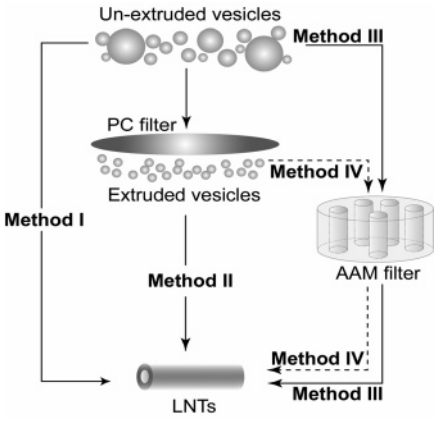

Figure 1. Schematic diagram for the preparation of LNTs by four different methods.

technique,[24,25] we prepared uniform lipid vesicles, which then self-assembled into LNTs with well-defined dimensions within the nanopores of an anodic alumina membrane (AAM) filter.

Experimental Section

Preparation of Uniform Vesicles with Thin Walls Using an Extrusion Technique. The glycolipid *N*-(11-*cis*-octadecenoyl)-β-D-glucopyranosylamine (**1**) used in this study has been detailed elsewhere.[17,26] Large multilamellar vesicles (MLVs) were prepared by vortexing the lipid **1** (1 mg) in distilled water (20 mL) at 96 °C for 2 h. The MLVs usually exhibit broad size distribution. They were forced through a serial of polycarbonate (PC) filters (Isopore Membrane Filters, Nihon Millipore K.K, Tokyo, Japan) with decreasing pore size from 5 μm to 100 nm under a relatively low pressure, approximately 5 kg/cm^2. For each filter, the extrusion process was repeated two times using a stainless steel extruder (Lipex Biomembranes, Inc., Vancouver, B.C., Canada), which was immersed in a water bath (90 °C) to make the extrusion performed at temperatures above the gel-to-liquid crystalline phase transition temperature (approximately 71 °C).

Formation of LNTs within the Nanopores of an AAM Filter. The AAM filter of 25 mm in diameter was commercially available from Whatman Japan KK (Tokyo, Japan). It possesses straight-through pores of 200 nm in diameter. The aqueous suspension of the lipid vesicles was loaded into the AAM nanopores at 90 °C using the same extruder as used for the vesicle extrusion. One piece of AAM filter was placed at the bottom of the extruder. A 10 mL aqueous suspension of vesicles was added into the cylinder of the extruder. A constant pressure (approximately 5 kg/cm^2) was applied to force the suspension through the AAM filter. After the majority of the suspension passed through the filter and a small amount of suspension still remained in the extruder, the pressure was stopped to keep the suspension staying within the nanopores. The water bath was then switched off and the AAM filter containing the lipid vesicles and the bulk aqueous suspension were cooled to room temperature. The LNTs formed both in the nanopores and the bulk aqueous suspension. Figure 1 shows four different methods to produce LNTs (method I: in-bulk self-assembly of unextruded vesicles; method II: in-bulk self-assembly of extruded vesicles; method III: in-pore self-assembly of unextruded vesicles; method IV: in-pore self-assembly of extruded vesicles).

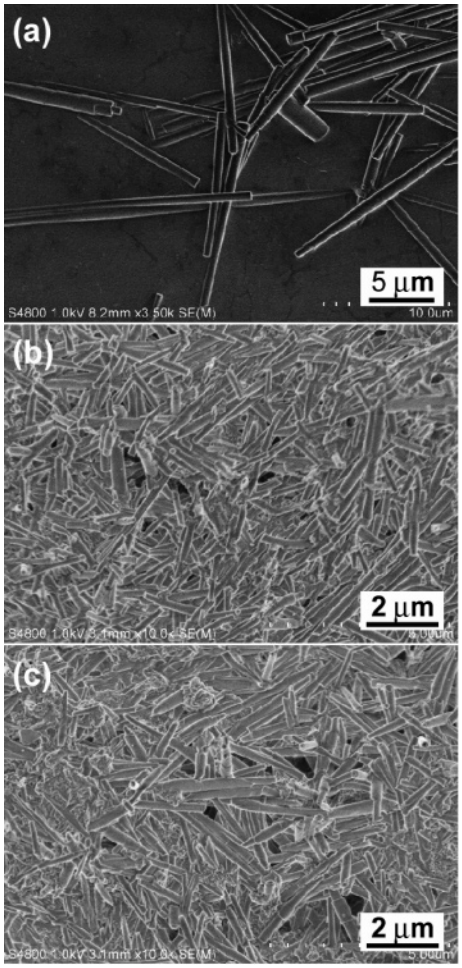

Figure 2. SEM images of the LNTs prepared by (a) method I, (b) method III, and (c) method IV.

Removal of the AAM Filter and Collection of the LNTs. After the temperature of the extruder and the bulk suspension became the same as room temperature, the AAM filter was taken out of the extruder. The surface of the filter was flushed by distilled water to wash away the attached LNTs formed outside the nanopores. The polypropylene support ring of the AAM filter was cut away. Then the AAM filter was dissolved away in 1 mol L^{-1} NaOH solution. The LNTs formed within the nanopores were collected by centrifugation at 3000 rpm for 10 min. After the removal of the supernatant, the concentrated LNT suspension at the bottom of the centrifuge tube was collected.

Scanning Electron Microscope (SEM) and Scanning Transmission Electron Microscope (STEM) Observations. A 5 μL suspension of the LNTs was dropped onto a transmission electron microscope (TEM) grid and a double-sided tape was glued to a SEM grid. The samples were dried in air and then in a vacuum to remove the water. SEM and STEM (FE-SEM S-4800, Hitachi High-Technologies Corp., Japan) observations were carried out at 1 and 30 kV, respectively. The dimensions of the obtained LNTs were determined by measuring the diameters on SEM and STEM images. The wall thickness of the LNTs was calculated based on the measured outer and inner diameters. The dimensions of the LNTs prepared by four different methods (methods I, II, III, and IV) were compared with one another. For each method, more than 60 LNTs were measured to give dimension distribution and mean dimensions.

(24) Olson, F.; Hunt, C. A.; Szoka, F. C.; Vail, W. J.; Papahadjopoulos, D. *Biochim. Biophys. Acta* **1979**, *557*, 9.
(25) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 55.
(26) Guo, Y. L.; Yui, H.; Minamikawa, H.; Masuda, M.; Kamiya, S.; Sawada, T.; Ito, K.; Shimizu, T. *Langmuir* **2005**, *21*, 4610.

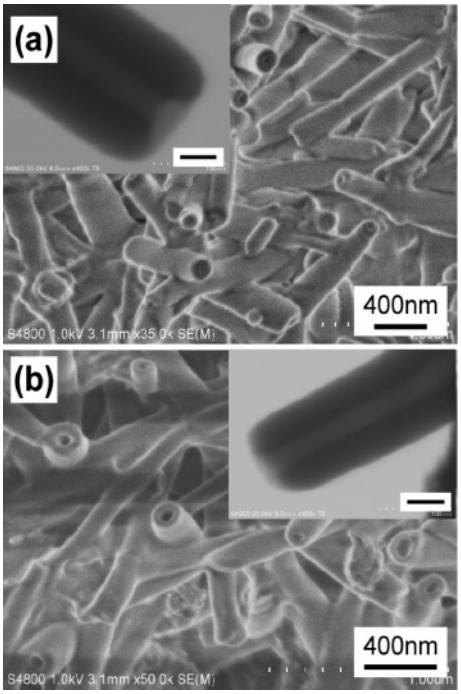

Figure 3. Higher magnification SEM and STEM images of the LNTs prepared by (a) method III and (b) method IV. The scale bars in both STEM images are 50 nm.

Table 1. Mean Outer Diamer, Inner Diameter, and Wall Thickness of the LNTs Prepared by Four Different Methods

	outer diameter (nm)	inner diameter (nm)	wall thickness (nm)
method I	392	61	145
method II	295	43	124
method III	204	56	74
method IV	148	52	48

Results and Discussion

STEM and SEM Images of the LNTs Formed within the Nanopores of AAM Filters. Figure 2 shows the LNTs prepared by three different methods. The LNTs self-assembled in the bulk aqueous dispersion containing unextruded vesicles (method I) have relatively large and multidisperse outer diameters ranging from a few hundred nanometers to a few micrometers (Figure 2a), whereas the LNTs formed within the AAM nanopores (methods III and IV) have much smaller and uniform outer diameters (Figures 2b and 2c). The outer diameters, approximately 200 nm, correspond to the pore size of the AAM filter used. This means that the confinement by the nanopores caused the decrease in distribution of outer diameters. SEM images show that the LNTs self-assembled from the extruded vesicles (method IV, Figure 2c) possess similar dimensions as those formed from the unextruded vesicles (method III, Figure 2b). However, higher magnification SEM images revealed that the wall thickness of the LNTs grown from the extruded vesicles (method IV, Figure 3b) decreases as compared to that of LNTs formed from the unextruded vesicles (method III, Figure 3a). Corresponding STEM images (inset at the corners of the SEM images) clearly show the difference in wall thickness between the two types of LNTs. The LNT prepared by nethod III is 191, 42, and 74 nm in outer diameter, inner diameter, and wall thickness, respectively, whereas the LNT by method IV has smaller dimensions, 123, 30, and 46 nm, respectively. Not only the wall thickness but also the outer and inner diameters were found to decrease when the extrusion technique was employed.

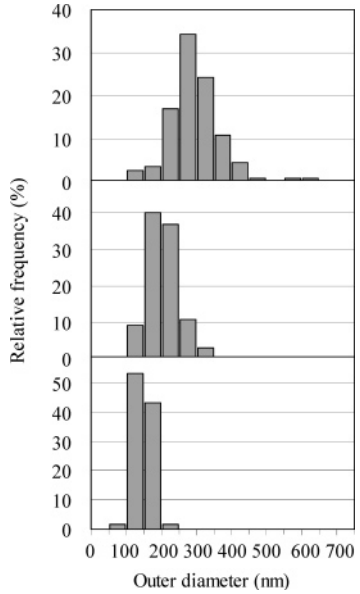

Figure 4. Histograms of outer diameters of the LNTs prepared by (a) method II, (b) method III, and (c) method IV.

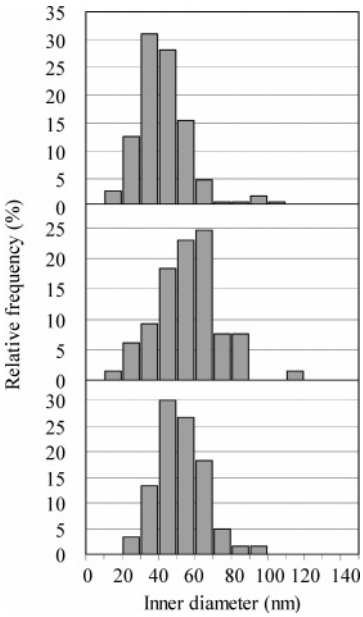

Figure 5. Histograms of inner diameters of the LNTs prepared by (a) method II, (b) method III, and (c) method IV.

Dimension Distribution of LNTs. To clarify the effects of extrusion and nanopore confinement, we compared the dimension distribution and mean dimensions of the LNTs prepared by the four different methods. Figures 4, 5, and 6 show histograms of outer diameters, inner diameters, and wall thickness, respectively. Table 1 summarizes mean outer diameter, inner diameter, and wall thickness of the LNTs prepared by four different methods.

The outer diameters of the LNTs prepared by method II have a wide distribution ranging from 100 to 650 nm, whereas the distribution of outer diameters for the LNTs grown in the AAM nanopores (methods III and IV) greatly decreased (Figure 4). This indicates that the use of AAM

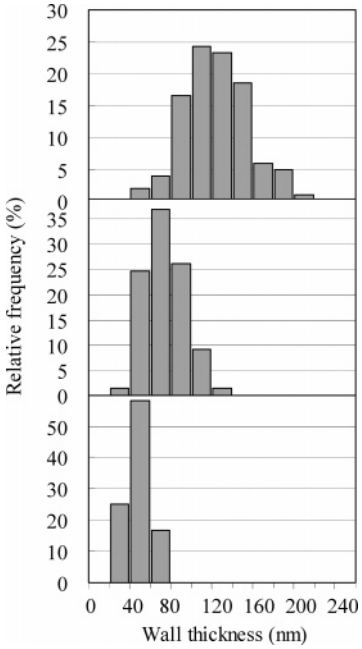

Figure 6. Histograms of wall thickness of the LNTs prepared by (a) method II, (b) method III, and (c) method IV.

nanopores can effectively decrease the outer diameter distribution due to its confinement effect. The mean outer diameter of the LNTs prepared by method III is 204 nm, corresponding to the pore size of the AAM filter. That of the LNTs prepared by method IV is 148 nm, much smaller than the pore size of the AAM filter, but it approaches the pore size of the extrusion filter (Table 1). This indicates that the pore size of the PC filter used for extrusion directly affected the outer diameters of the LNTs prepared by method IV.

The successive use of the extrusion technique and AAM nanopores (method IV) also decreased the distribution of inner diameters (Figure 5) and wall thickness (Figure 6). The mean inner diameter and wall thickness of the LNTs prepared by method IV are smaller than those by method III (Table 1). Especially, the wall thickness of the LNTs prepared by method IV is well-defined. It is 48 nm, much smaller than that of LNTs prepared by a single use of AAM nanopores (method III, 74 nm), indicating the extrusion effectively reduced the wall thickness of the LNTs.

Figure 7 shows the possible formation mechanism for dimension-controlled LNTs prepared by method IV. A low pressure forces the multidisperse vesicles through the 100 nm PC filter to prepare uniform vesicles with thin walls.[24,25]

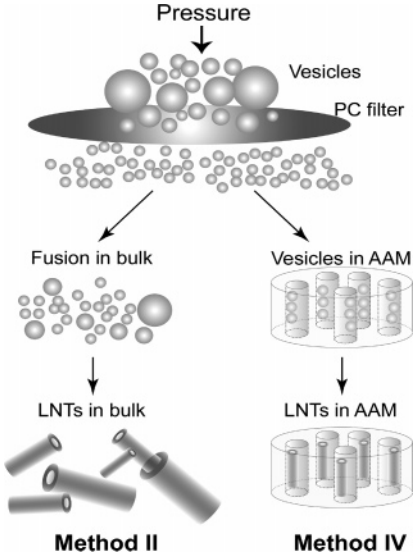

Figure 7. Schematic diagram of the possible mechanisms for the formation of LNTs, on the left side: prepared by method II; on the right side: prepared by method IV.

The outer diameters of the extruded vesicles are reduced to approximately 100 nm. However, in the bulk suspension, the extruded vesicles are unstable and tend to fuse into relatively large vesicles before the formation of tubular structures. The fusion results in the formation of large-sized LNTs, as well as a wide dimension distribution. On the other hand, when the extruded vesicles are loaded into the 200 nm AAM nanopores, the confinement by nanopores proved to obstruct the fusion process to form relatively large vesicles. The extruded vesicles will arrange along the inner surfaces of the nanopores and eventually fuse into LNTs, which have the dimensions similar to those of the extruded vesicles.

Conclusion

We prepared LNTs with defined dimensions by successive use of an extrusion technique and AAM nanopores. This method effectively decreased the dimension distribution of the LNTs. The mean outer diameter, inner diameter, and wall thickness were 148, 52, and 48 nm, respectively, determined by the pore size of the extrusion PC filter and the confinement of AAM nanopores.

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