

論文内容の要旨

論文題目 Dimension-Controlled Synthesis and Nanoscopic Analyses of Self-Assembled Glycolipid Nanotubes

(自己組織化糖脂質ナノチューブのサイズ制御及び局所分析)

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Self-assembled lipid nanotubes with hydrophilic surfaces, composed of biocompatible lipid molecules, have potential use in biomedical and biotechnological areas.^{1,2} Our group has developed self-assembled glycolipid nanotubes (LNTs) composed of *N*-(11-*cis*-octadecenoyl)- β -D-glucopyranosylamine (1) (Figure 1 bottom). Glycolipid 1 form vesicles in water above the gel-to-liquid crystalline phase transition temperature (71 °C). Upon cooling, the vesicles spontaneously grow into tubular structures several hundred nanometers in diameter.³

Since the surfaces of the Glycolipid 1-formed LNTs are covered with sugar groups, one proposed application of such LNTs is as nanofluidic channels for single molecular separation and detection of biomacromolecules (Figure 1 top). At present, typical top-down fabrication

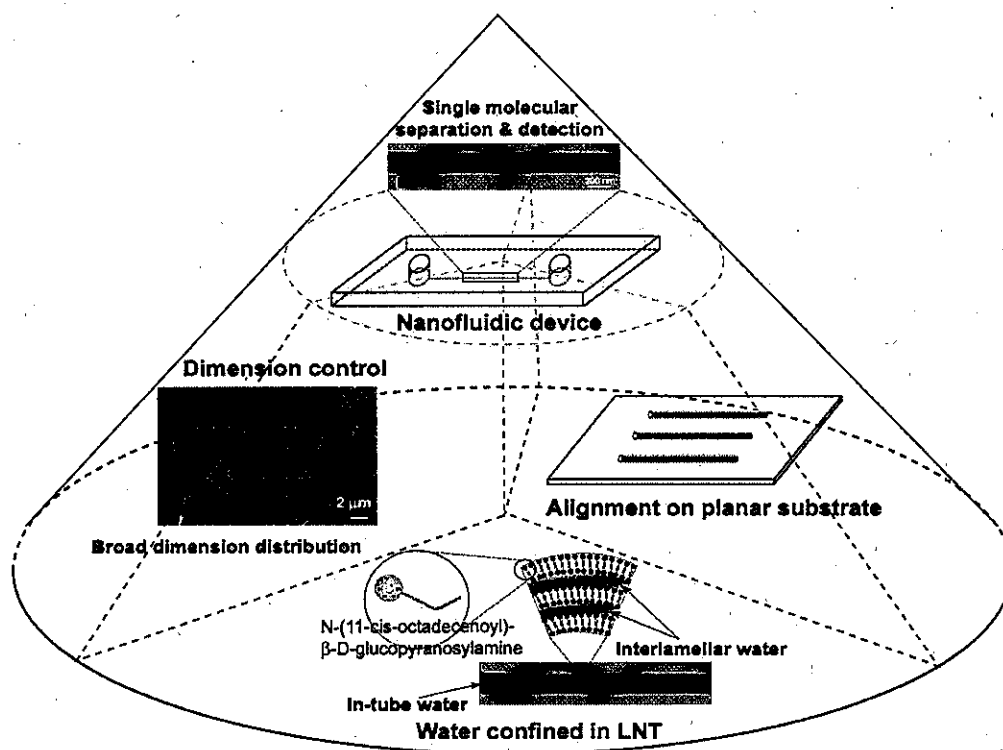


Figure 1. Application of the self-assembled LNTs to nanofluidic devices.

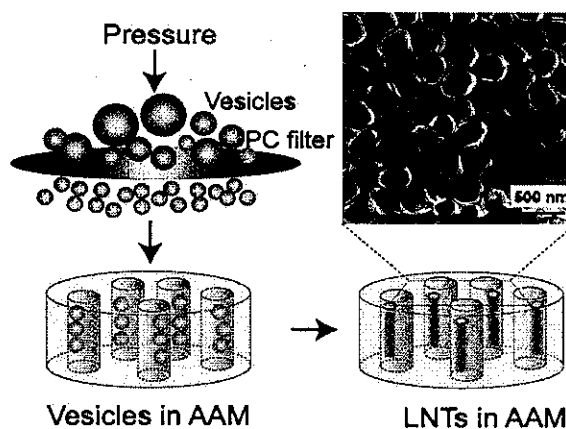
methods for nanofluidic channels including electron beam lithography and ion beam milling techniques are complicated and costly. Moreover, there is a limit of minimum size, approximately 50 nm. On the other hand, self-assembled LNTs provide a simple approach to fabricating nanofluidic channels with diameters ranging from a few to several hundred nanometers. However, for practical applications to nanofluidic devices there are still some

problems with the LNTs such as a wide dimension distribution and the lack of alignment technique (Figure 1).

To develop the self-assembled LNTs into nanofluidic devices for single molecular separation and detection, we first created a novel method to produce the LNTs with defined dimensions. Furthermore, we developed a Two-Step Microextrusion technique to align the LNTs with a few hundred nanometer diameters. Finally, during the study on the self-assembly properties of the LNTs, We found water confined within the walls of the LNTs plays an important role in stabilizing the tubular structure and determining the physical properties. To understand the structure of the LNTs, we investigated the local aqueous environment of the LNTs.

Synthesis of Dimension-Controlled LNTs

Multi-wall LNTs consisting of Glycolipid 1 generally exhibit broad dimension distribution (Figure 1). Several thermodynamic methods allowed us to prepare LNTs with uniform diameters.³ 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. However, preparation of the LNTs with defined inner diameter and wall thickness is still difficult even if one can employ this template method.⁴ Here, we developed a simple and effective method to prepare LNTs with defined dimensions including outer, inner diameters and wall thickness (Figure 2). Since nonuniform-sized spherical vesicles are the possible reason for a wide dimension distribution of the LNTs, to decrease the size distribution of the vesicles, in this method we applied a



Vesicles in AAM **LNTs in AAM**
Figure 2. Dimension control of the LNTs by successive use of vesicle extrusion and porous template.

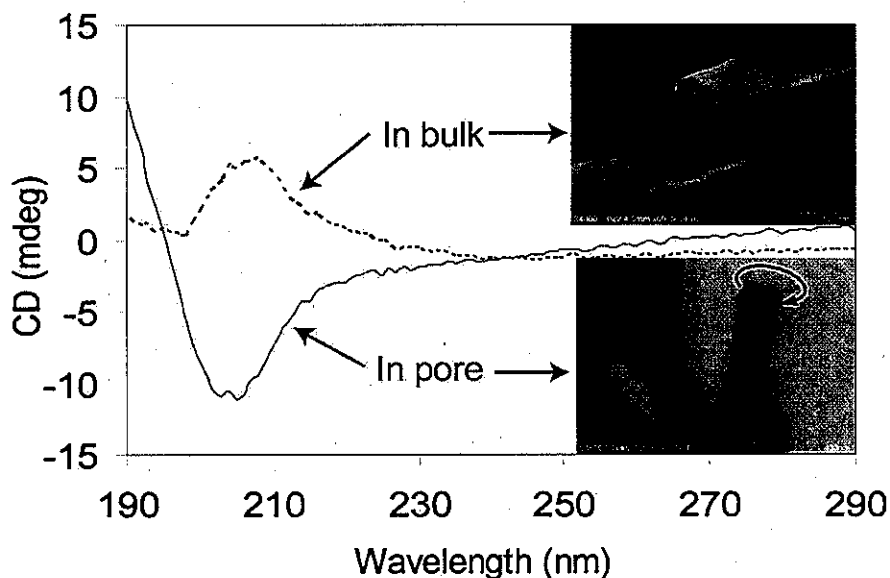


Figure 3. CD spectra and EM images of the LNTs in pore and in bulk.

conventional vesicle extrusion technique to produce the uniform vesicles. Furthermore, to avoid the fusion of the extruded vesicles into the larger and nonuniform ones, the vesicles were loaded into the straight-through cylindrical nanopores of an anodic alumina membrane (AAM) filter. The vesicles grew into the LNTs within the nanopores of the AAM. Successive use of extrusion technique and porous template produced the LNTs with defined dimensions. An attempt was also made to control the dimensions of the LNTs by changing the pore size of the extrusion filters.

The theory for the self-assembly process in the bulk solution has been discussed by Selinger and colleagues.⁵ However, a self-assembly process within nanopores should be different. The structural and physical properties of the LNTs formed in nanopores were investigated based on Scanning Electron Microscopy (SEM), Scanning Transmission Electron Microscopy (STEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Circular Dichroism (CD) and Differential Scanning Calorimetry (DSC). These results were compared to those of the LNTs in bulk. According to the CD and DSC results, we found in-pore self-assembled LNTs possess more stable molecular packing with opposite handedness (Figure 3). We proposed a different mechanism for in-pore self-assembly that the vesicles loaded into the nanopores fuse into nanotubes due to the nanopore confinement.

Alignment of the LNTs on Planar Glass Substrate Using Two-step Microextrusion Technique

For a further application, demands for technology of manipulation and alignment of nanoparticles are increasing. External electric, magnetic and electromagnetic field have been successfully employed to spatially manipulate and align nanoparticles of various shapes and sizes. However, such manipulation and alignment are greatly dependent on the electric, magnetic and optical properties of nanomaterials. Moreover, the external electric, magnetic or optical field usually causes damage to the nanoparticles.

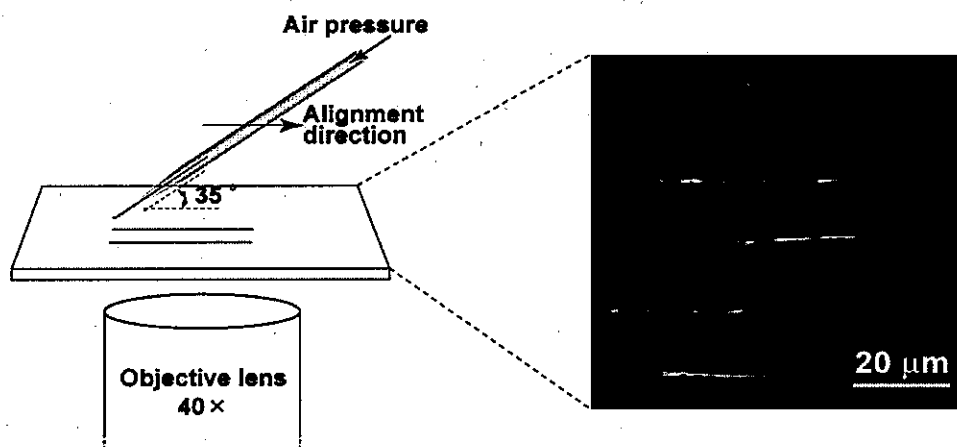


Figure 4. Alignment of the LNTs on planar glass substrate.

Our group has developed a technique to align LNTs 50 nm in diameter on planar substrate. Base on this technique, we developed a Two-step Microextrusion technique to align relatively large nanofibers and nanotubes. Using this technique, we successfully aligned the LNTs (200 nm in diameter) onto planar glass substrate. The optical microscopic image shows the aligned LNTs tens of micrometers in length are parallel to one another (Figure 4).

Study on Interlamellar Water Confined in LNT Walls

Water confined between the bilayer membranes (interlamellar water) play an important role in determining the structural stability and physical properties of the LNTs (Figure 5). To understand the structure and physical properties of the LNTs, we studied interlamellar water using X-ray diffraction (XRD) and Fourier Transform Infrared spectroscopy (FTIR).

Based on XRD measurement, I detected the average thickness of interlamellar water was approximately 1.3 nm. FTIR study revealed hydrogen bonds in interlamellar water were strengthened by the sugar headgroups, although the extent of hydrogen bonding was decreased due to geometrical confinement. Formation of stronger hydrogen bonds in interlamellar water effectively stabilized the multilamellar tubular structure.

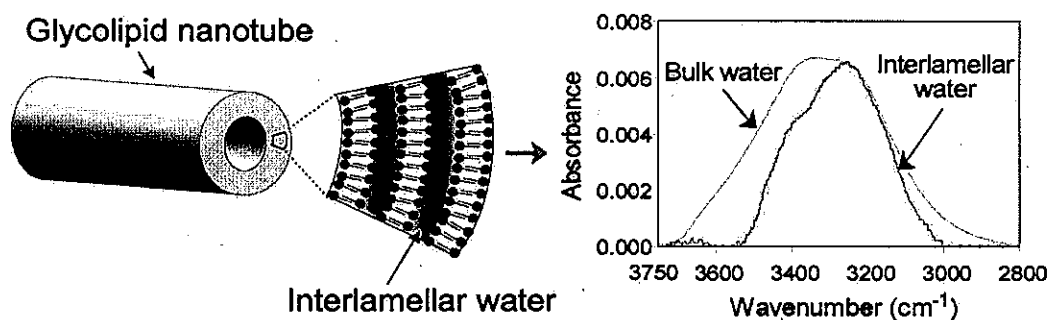


Figure 5. ATR-FT-IR spectra of interlamellar water and bulk water.

Conclusions

Successive use of vesicle extrusion and porous template synthesized dimension-controlled LNTs to meet various requirements for nanofluidic applications. Two-step Microextrusion technique successfully aligned the Glycolipid 1-formed nanotubes on planar glass substrate. This manipulation technique is to be used to incorporate the LNTs into nanofluidic devices. Interlamellar water stabilizes the multilamellar tubular structure by strongly hydrogen bonding. The finding helps to understand the structural stability of the LNTs in aqueous solutions containing some salt solutes. Dimension-controlled synthesis, alignment of the LNTs and nanoscopic analyses provide theoretical basis and technique tools for the further applications to biomedical and biotechnological areas, especially to nanofluidic devices. In the future, I plan to align the dimension-controlled LNT between the microelectrodes to develop a nanofluidic device for single molecular separation and detection.

References

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