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[18] The sonication was proved not to degrade either the copolymers or α-CD, as evident by means of ^{1}H NMR spectroscopy, GPC, and TLC. Also see ref. [6].

Lipid Nanotubes

Aligning a Single-Lipid Nanotube with Moderate Stiffness**

Hiroshi Frusawa, Akihiro Fukagawa, Yuki Ikeda, Jun Araki, Kohzo Ito, George John, and Toshimi Shimizu**

Many types of amphiphilic molecules have been found to self-assemble into cylindrical tubules in aqueous solutions.[1–12] The synthetic lipid tubules can provide intriguing hydrophilic internal and external surfaces, unlike carbon nanotubes,[13] and therefore they have unique potential not only as cytomimetic tubules but also as hollow nanospaces for chemical reactions, the transferral of biomolecules, etc. The mechanical properties of lipid nanotubes, however, have not been investigated to date, in contrast to extensive studies on microtubules[14,15] or carbon nanotubes.[16] We have measured the Young's modulus of a single-lipid nanotube that consists of renewable-resource-based synthetic glycolipids (cardanyl-β-D-glucopyranoside)[17] by using optical tweezers. Herein we report that the Young's moduli of the present lipid nanotubes are similar to those of microtubules[15] with outer and inner diameters of the same order. Furthermore, because of the moderate stiffness, we have succeeded in aligning the single-lipid nanotube on a glass plate by microextrusion of the aqueous dispersion.

To measure accurately the flexural rigidity of the single nanotube, we should pay careful attention to the aggregation in an aqueous dispersion. Figure 1 is a transmission electronic microscopy (TEM) image which shows that the lipid nanotubes are completely isolated and form no bundles. TEM images of higher resolution have further shown that the outer and inner diameters are 50 and 10 nm, respectively,[17] which is similar to the values (25 and 10 nm) of microtubules.[18]

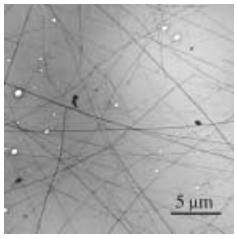

Figure 1. TEM image of lipid nanotubes in an aqueous dispersion, which were used for flexural rigidity measurements.

A drop of the aqueous dispersion was placed on a glass slide for optical traps, and pressed between coverslip and slide. Because some nanotubes were thereby forced to adhere firmly to the substrate, we had no difficulty in finding a target fixed well at only one end. Using optical tweezers (Sigma Koki LMS-46755), the monoattached nanotube was bent as follows: A laser beam was focused onto the target, the lipid nanotube was trapped in the focal point in a similar way to microtubules.[14,15] This trapping occurs because of the difference in refractive index between the nanotube and the surrounding medium, water. Therefore, while it is possible to manipulate the tubule in a direction perpendicular to its long axis, we cannot do this along the long axis, except by manipulation at either end. We can thus bend the nanotubes by moving the stage and capturing the tube near the free end.

The laser beam was switched off after some bending had occurred, and the resulting bow-shaped nanotube started to relax to its initial straight form. From the relaxation time τ, we can evaluate the flexural rigidity K, because the balance condition between elastic and hydrodynamic force[15] yields the time-dependence of relaxation of the free end, as shown in Equations (1) and (2). Here, y is the ordinate perpendicular to the initial straight line, L is the contour length, η is the viscosity, and d is the outer diameter.

$$y(L,t) = y(L,0)\exp(-t/\tau) \quad (1)$$

$$\tau = \frac{11\pi\eta L^4}{60\,\mathrm{K}\ln(L/2\,\mathrm{d})} \quad (2)$$

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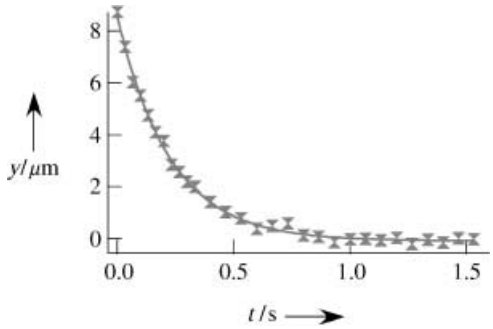

Figure 2. Time course of the free end relaxing from its bent position to the initial point. Closed circles represent a result obtained by image processing, and the solid line shows a fit to the exponential decay, that is, Eq. (2).

Figure 2 is a plot of $y(L, t)$ versus time for the free end of the tube, measured at 23 °C. Fitting of Eq. (2) to the curve gives the relaxation time $\tau \approx 0.22$ s, from which we obtain the bending rigidity $K \approx 2.2 \times 10^{-22}\,\mathrm{N\,m^2}$. In the evaluation, we assume that the viscosity η mainly comes from the solvent, water, and ignore the friction between nanotube and slide. We also evaluated the stiffness of other nanotubes using the water viscosity, and obtained almost identical values to the first value, which supports the above assumption.

To compare the present lipid nanotube with microtubules, we first converted the bending stiffness K into the Young's modulus $E = K/I$. Here I is the geometrical moment in inertia and is given for the tubes as $I = (d_o^4 - d_i^4)\pi/64$ with outer diameter d_o and inner diameter d_i. The evaluation gives $E \approx$ 720 MPa for the present nanotube, and $E \approx 1000$ MPa for microtubules.[14] We therefore conclude that the glycolipid nanotube is a cytomimetic tubule, in that both the elasticity and the diameter size are similar to those of microtubules. Our lipid nanotube and microtubules differ, however, in their persistence lengths L_p, which measures the crossover from a rodlike to a flexible form. Estimating from the relation $L_p = K/k_BT$, we have $L_p \approx 5$ cm for the lipid nanotube, which is longer than those of microtubules, $L_p \approx 0.1$ cm.[14] The difference is ascribable to a thicker (10–15 nm) cylinder wall for the present nanotubes than the microtubules: because the wall thickness is twice as large as that of microtubules, the persistence length is not identical, but the Young's modulus is similar.

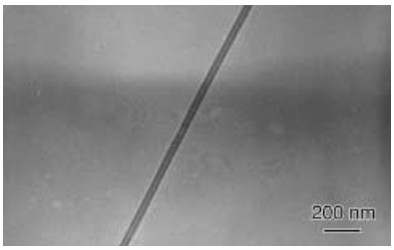

Figure 4. TEM image of a single lipid nanotube which is aligned by our manipulation method onto Formvar (poly(vinyl formal)) film spread on a copper grid.

Considering that our nanotubes have modest Young's moduli as well as long persistence lengths, one may envisage the possibility of aligning them. Indeed we demonstrate below that a microextrusion of aqueous nanotube dispersion is a simple method for directing single nanotubes.

Figure 3 illustrates the microextrusion process. We have exploited a microinjection system (eppendorf FemtoJet + InjectMan), and have used a dispersion with the same concentration as that of the above bending measurement; nanotubes are fully dispersed before microextrusion. The first step (Figure 3 a) involves touching the microneedle (500 nm inner diameter) lightly onto the slide, which allows a small amount of lipid nanotube dispersion to drop onto it. The water component evaporates away quickly because of the irradiation from the halogen lamp needed for microscopy, during which time an edge of the single nanotube adheres to the glass plate. We can hence freely draw a fine line with a single lipid nanotube, by moving the microneedle in an arbitrary direction. In practice the manipulation does not work well every time, because nanotubes are not always contained in the first drop; we sometimes inject only water which soon evaporates.

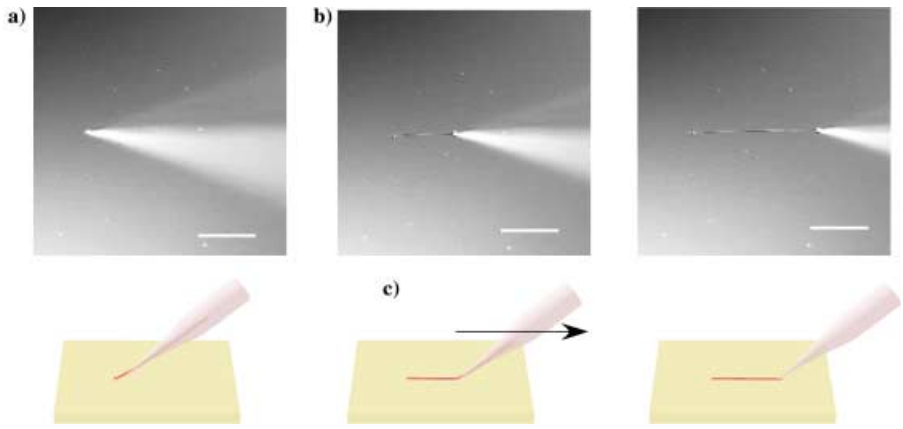

Figure 3. Confocal laser scanning micrographs (Carl Zeiss LSM510) of a fine line drawn with single nanotubes by microextrusion, and its explanatory schematics. The white triangles in the images correspond to the tip of the needle: a) the needle end touches a glass slide. because a positive pressure is applied against backward flow, even before injection, a very small amount of solution drops onto the glass. As a consequence, while water quickly evaporates, the nanotubes become attached; b) by slowly moving the needle linearly, nanotubes are extruded one after another; c) when the injection is stopped, the line ends.

Of course, it must be checked whether the microextruded line observed by optical microscopy is truly a single nanotube or not. To this end we drew a line using the above microextrusion technique onto Formvar (poly(vinyl formal)) film spread onto a copper grid. Figure 4 is the TEM image of this line, and shows that the line is indeed a single lipid nanotube. Remarkably, this

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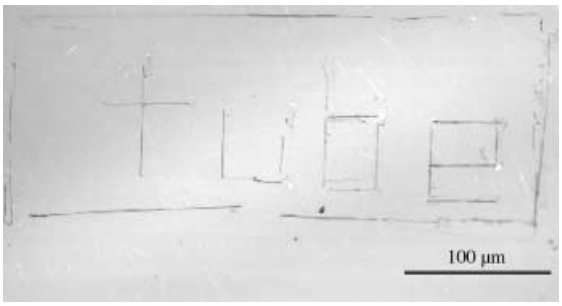

Figure 5. Confocal laser scanning micrograph of the word "tube" drawn with single nanotubes using our microinjection system. The frame, also drawn using single nanotubes, is 0.37 mm long and 0.15 mm wide. A higher magnification of this is shown in Figure 4.

manipulation easily aligns a single tube in an arbitrary direction. For demonstration we have further written the word "tube", as shown in Figure 5.

In summary, we found that the glycolipid nanotube could be a good candidate for a cytomimetic tubule in terms of its mechanical properties. Moreover, exploiting the moderate rigidity, we have developed a novel but simple aligning method: a fine line of the single nanotube is drawn freely simply by microextruding the aqueous dispersion on glass plate. Our manipulation methodology promises to open up fascinating possibilities for lipid nanotubes beyond only a substitute for microtubules; for example, a nanoneedle could be realized by extruding half of the single nanotube and fixing it at the tip of a microextrusion needle; also bridging or branching of the nanotubes on glass or metal substrates might be used as a mold for the formation of a wire and hub inside them, in molecular electronic devices; lastly, a single nanotube channel and its assembled array should provide 1D nanospace for the separation of advanced macromolecules beyond the submicro total analysis system (subμTAS).

Experimental Section

Preparation of single lipid nanotubes: These were prepared in water through self-assembly of renewable-resource-based, synthetic glycolipid cardanyl-β-D-glucopyranoside, which is a mixture of 1-*O*-3′-*n*-(8′(*Z*),11′(*Z*), 14′-pentadecatrienyl)-phenyl- β -D-glucopyranoside (ca. 29 wt%), 1-*O*-3′-*n*-(8′(*Z*),11′(*Z*)-pentadecadienyl) phenyl-β-D-glucopyranoside (ca. 16 wt%), 1-*O*-3′-*n*-(8′(*Z*)-pentadecenyl) phenyl-β-D-glucopyranoside (ca. 50 wt%), and 1-*O*-3′-*n*-(pentadecyl) phenyl-β-D-glucopyranoside (ca. 5 wt%), as reported elsewhere.[17] Nanotube structures with 10–15 nm inner diameter and high axial ratios were confirmed by TEM, field-emission scanning electron microscopy (FE-SEM), confocal laser scanning microscopy, and atomic force microscopy (AFM). On the basis of X-ray diffraction studies, the tubular membrane wall consists of three to four interdigitated lipid bilayers. Combination of a hydrogen-bond network between the glucose headgroups, π–π stacking interactions between phenyl rings, and hydrophobic interactions between long alkyl chains are responsible for the stabilization of noncovalently formed nanotube architectures.

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Metal-Based Polyrotaxanes

Channels and Cavities Lined with Interlocked Components: Metal-Based Polyrotaxanes That Utilize Pyridinium Axles and Crown Ether Wheels as Ligands**

*Gregory J. E. Davidson and Stephen J. Loeb**

The formation of polyrotaxanes by metal–ligand self-assembly is a viable method for the programmed organization of mechanically linked molecular components into a repeating framework. This has significant potential for producing materials that contain functional molecular entities that may be addressable or controllable.[1] The challenges of designing, synthesizing, and crystallizing such materials are many and

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