

Size-Controlled Self-Assembly of Peptide Nanotubes Using Polycarbonate Membranes as Templates

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Peptide nanotubes have been self-assembled from the peptide monomers with polycarbonate membranes in solution to control the diameter of the nanotubes by using the membranes as templates. The diameters of self-assembled peptide nanotubes can be controlled by the pore sizes of the polycarbonate membrane templates, whereas peptide nanotubes that were not grown in the membrane pores were not monodisperse. Therefore, those nanotubes grown outside the membranes had to be removed before dissolving the templates in order to obtain monodisperse peptide nanotubes. The peptide nanotubes were self-assembled in the membrane pores as small as 50 nm in diameter.

Introduction

Assembly of nanometer-scaled building blocks into device configurations is an intensely investigated research field in nanotechnology.^{1–6} Because various nanowires have been applied as building blocks to develop electronics and sensors,^{7–12} production of uniform-sized nanowires becomes crucial for their real world applications as their electric and magnetic properties are sensitive to their sizes in the nanometer scale.^{13,14} To achieve the size selection of nanotubes, two approaches have been examined. One approach is to discriminate a certain length of nanotubes from the polydisperse nanotube-containing solutions using various separation methods.^{15–17} Another approach is to grow nanotubes monodisperse in diameter by using

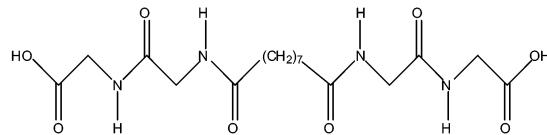


Figure 1. Chemical structure of the peptide monomer in order to assemble peptide nanotubes.

porous templates^{18,19} or by forming nanotubes on catalytic nucleation sites whose surface areas control the nanotube diameters.^{20,21}

One of the peptide bolaamphiphile molecules, bis(*N*- α -amido-glycylglycine)-1,7-heptane dicarboxylate (Figure 1), can be self-assembled into crystalline tubular structures in diameters of 20 nm – 1 μ m in a pH 5.5 solution.²² This peptide nanotube has been functionalized with metals,²³ proteins,²⁴ nanocrystals,²⁵ and porphyrins²⁶ for electronics and sensor applications. The peptide nanotubes were also immobilized onto substrates using hydrogen bonds²⁷ and biological recognitions.¹² Although the peptide nanotubes reveal those interesting properties, the polydisperse nature of the nanotubes in size has been a disadvantage for the above

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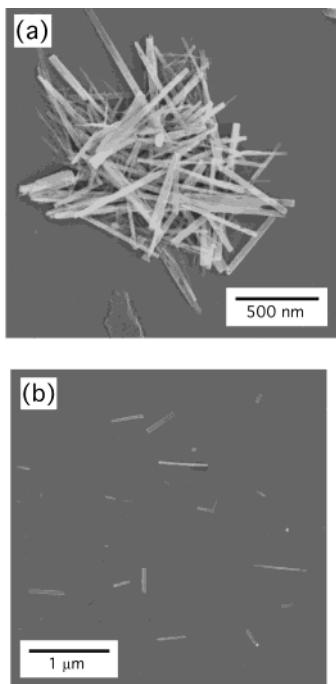


Figure 2. (a) Scanning electron micrograph of the templated peptide nanotubes aggregated after drying the solution containing high concentration of the peptide nanotubes; (b) Scanning electron micrograph of the templated peptide nanotubes dispersed after drying the solution containing low concentration of the peptide nanotubes. Pore size of the polycarbonate membrane template is 50 nm.

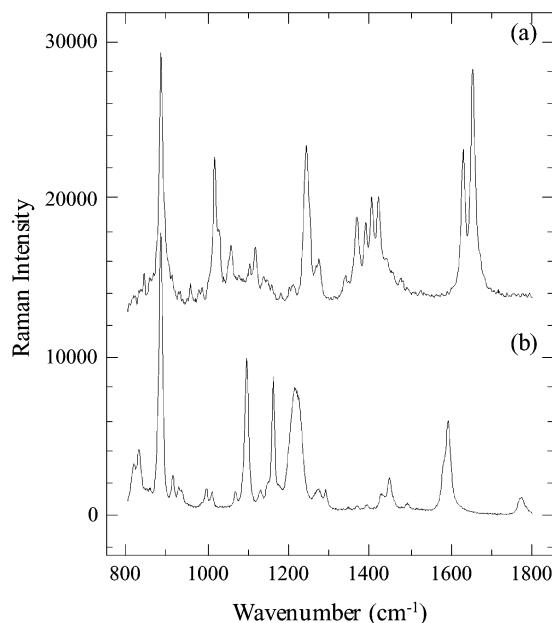


Figure 3. Raman spectra of (a) templated peptide nanotubes and (b) neat polycarbonate membranes.

applications.²⁴ In this report, we describe a method to prepare monodisperse peptide nanotubes using porous templates. The peptide monomers were self-assembled in polycarbonate membrane template-containing solutions, and the resulting peptide nanotubes that self-assembled in the pores were relatively monodisperse in diameter. We have also shown that the diameters of self-assembled peptide nanotubes can be controlled by the pore sizes of the polycarbonate membrane templates.

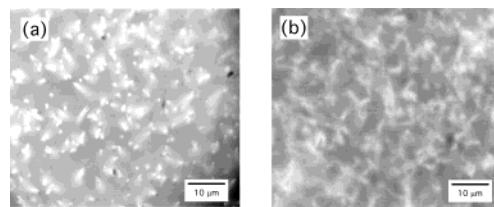


Figure 4. Light micrographs of (a) neat polycarbonate membrane and (b) peptide nanotubes grown on polycarbonate membrane.

Whereas various nanotubes have been synthesized in membranes to control their dimensions,^{18,19,28–30} there are relatively few reports on controlling the shape of self-assembled organic molecules by using porous templates without external electric fields and catalysts.

Experimental Section

The peptide monomer bis(*N*- α -amido-glycylglycine)-1,7-heptane dicarboxylate was synthesized as described elsewhere.³¹ Polycarbonate microporous membranes with nominal pore diameters of 10, 30, 50, 80, 100, and 500 nm, and 1 μ m, whose pore size distributions are $\pm 0\%$ and $\pm 20\%$, were obtained from Osmonics. The template membrane was immersed into a solution of the peptide monomer prepared by adding 23.2 mg of the monomer to 6 mL of 30 mM NaOH. The pH was subsequently adjusted to 5.5 by the addition of 50 mM citric acid. The resulting solution containing the membrane stood at room temperature. After two weeks without disturbance, the peptide monomers were self-assembled into a tubular structure and the peptide nanotubes appeared in the membrane pores and on the membrane surfaces. To remove the nanotubes on the membrane surfaces, the membranes containing the peptide nanotubes were ultrasonicated for 5 min and washed by water. The template membranes were then dissolved by immersing them in 3 mL of CH_2Cl_2 / $\text{C}_2\text{H}_5\text{OH}$ (9:1) solution for 3 h. The absence of $\text{C}_2\text{H}_5\text{OH}$ would result in rapid dissolution of the polycarbonate membranes. After adding 5 mL of CH_2Cl_2 into this solution, the mixture was ultrasonicated for 30 min to completely dissolve the template membranes. The solvents were evaporated at room temperature after the ultrasonication and then 10 mL of deionized water was added to the dried samples. These solutions were ultrasonicated again for another 30 min. Those solutions were microcentrifuged at 13 000 s^{-1} for 2 min to collect the peptide nanotubes.

Scanning electron micrographs of the peptide nanotubes and the membranes were obtained by a JEOL JSM-6400 F with 10 kV of accelerating voltage. The nanotube and membrane samples were dried on silicon grids in a vacuum chamber overnight, and then Au/Pt was sputtered and coated onto these samples. Raman spectra were acquired with a LabRam (Jobin Yvon/Horiba, Edison, NJ) confocal Raman microscope. Glass slides were rinsed with hexane, and the washed samples were deposited onto the slides and dried at room temperature. The 632.8 nm emission from an integrated He–Ne laser was spatially filtered and then injected into an integrated Olympus BX40 microscope by a holographic notch filter. A 100 \times working distance objective focused the beam to provide approximately 6 mW in a spot of less than 1 μ m diameter. The Raman scattering was collected at 180° geometry by the focusing objective, and the reflected excitation laser was rejected by a notch filter. The filtered radiation was focused at the entrance slit (250 μm) of a 0.3-mm spectrograph. An 1800 gr/mm grating

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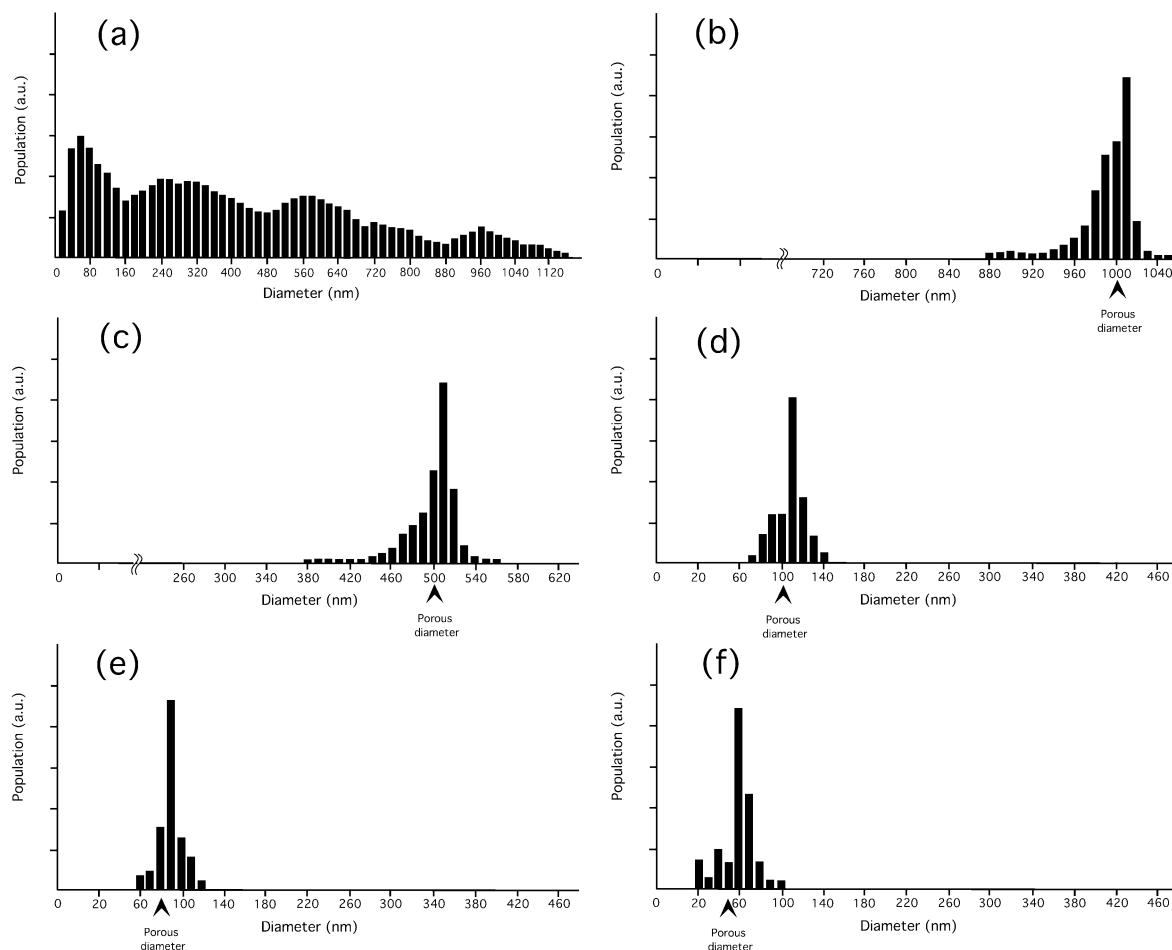


Figure 5. Diameter distributions of templated peptide nanotubes grown in (a) suspension; (b) 1- μ m membrane; (c) 500-nm membrane; (d) 100-nm membrane; (e) 80-nm membrane; and (f) 50-nm membrane. The polycarbonate membrane pore sizes are marked with arrows.

dispersed the spectrum across a 1024×256 pixel TE-cooled CCD detector, providing a dispersion of approximately $1 \text{ cm}^{-1}/\text{pixel}$.

Results and Discussion

After the peptide nanotubes were self-assembled with the polycarbonate membranes for 2 weeks in solution, the peptide nanotubes grown in the membrane pores were extracted by dissolving the membranes with CH_2Cl_2 . Scanning electron micrographs of the template-synthesized peptide nanotubes grown in the 50-nm-pore polycarbonate membrane are shown in Figure 2. The peptide nanotubes were observed to aggregate when the solutions with high concentration of the peptide nanotubes were dried as shown in Figure 2(a). When the solutions were diluted and dried, the peptide nanotubes were dispersed and the nanotubes could be isolated, as shown in Figure 2(b). To confirm that those templated nanotubes were not fragments of the membrane residue, we compared vibrational spectra of the neat polycarbonate membranes and the peptide nanotubes after dissolving the polycarbonate membrane (Figure 3). The vibrational modes that appeared in the membrane spectrum (Figure 3(a)) were not observed in the nanotube spectrum (Figure 3(b)). This spectral mismatch indicates that the templated peptide nanotubes obtained after removing the polycarbonate membranes were not fragments of the membranes. In fact, the peptide

nanotube spectrum in Figure 3(b) exactly matches the spectrum of peptide nanotubes grown in suspension without templates.²²

When the peptide monomers were self-assembled with the porous polycarbonate templates in a pH 5.5 solution the tubular assemblies appeared on the membrane surfaces after 2 weeks. Figure 4 (a) is a light micrograph of the 1- μ m polycarbonate membrane, and Figure 4(b) is the same membrane after the peptide nanotubes were self-assembled. Although this image was not sharply focused because parts of the peptide nanotubes were grown perpendicular to the membrane surface, disappearance of the pores on the membrane surface that were observed in Figure 4(a), was clearly shown in Figure 4(b). But when the size distribution of these nanotubes was examined by the scanning electron microscope, they were still polydisperse (Figure 5 (a)) because the nanotubes grown in the suspension were also likely deposited on the membrane surfaces. Therefore, the polycarbonate membranes containing the peptide nanotubes were sonicated for 5 min and washed with water before examining the nanotube size distribution in each porous membrane in order to remove the nanotubes that were not grown in the membrane pores.

After those removals, the polycarbonate membranes were dissolved by CH_2Cl_2 to study the size distributions of the templated peptide nanotubes. The size distributions of the peptide nanotubes grown in 50-, 80-, 100-,

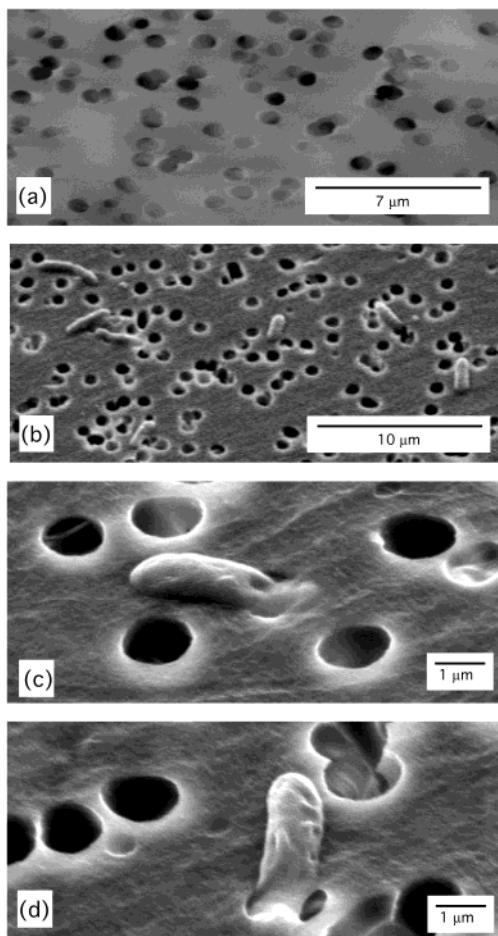


Figure 6. (a) SEM image of neat polycarbonate membrane template; (b) low-magnification SEM image of peptide nanotubes self-assembled in the template; (c) and (d) high-magnification SEM images of peptide nanotubes self-assembled in the template.

and 500-nm-, and 1- μm -, pore polycarbonate membranes are shown in Figure 5(b)–(f). Those histograms are based on the scanning electron micrographs of peptide nanotube samples, and the polycarbonate membrane pore sizes are marked with arrows in each figure. Figure 5(b)–(f) indicates a marked decrease in the polydispersity of the peptide nanotube diameters compared with that in Figure 5(a). The largest populations of grown nanotube diameters in Figure 5(b)–(f) also show strong correspondences with the membrane pore sizes. The peptide nanotubes removed from the membrane surfaces were polydisperse as Figure 5(a), which is consistent with our hypothesis. It should be noted that there were no peptide nanotubes detected in scanning electron micrographs or transmission electron micrographs in the 10- and 30-nm-pore polycarbonate membranes, whereas the smallest size of the nanotubes self-assembled in suspension without templates was observed at 20-nm diameter. This observation suggests that the

peptide monomers may not be able to self-assemble in the pore diameters near, or smaller than, 20 nm because of their geometric limitation of self-assembly in the cavity smaller than their natural growth size. The peptide monomer solution may not be able to fill the 30-nm or smaller membrane pores enough to initiate the self-assembly in the pores, which could also limit the nanotube growth.^{28,30}

Before dissolving the polycarbonate membrane by CH_2Cl_2 , we imaged the 1- μm pore membrane surface containing the peptide nanotubes (after washing the membrane surface) using the scanning electron microscope (SEM). Figure 6 (a) shows a SEM image of the neat polycarbonate membrane surface. Figure 6(b) shows a low magnification SEM micrograph of the peptide nanotubes grown in the membrane pores. Ultrasonication with water seemed to remove the majority of the peptide nanotubes deposited on the surfaces. Some of the nanotubes were bent on the membrane surface (Figure 6(c)) and some were grown perpendicular to the surface (Figure 6(d)), but all of them were associated with the pores. Whereas polymeric nanotubes,^{28,30} carbon nanotubes,³² and metal nanotubes^{33,34} can fill all membrane pores by incorporating nucleation sites in the pores, the self-assembled peptide nanotubes did not occupy all pores, as shown in Figure 6(b), because of the lack of nucleation sites in the pores.

Conclusions

We have shown that the diameters of self-assembled peptide nanotubes can be controlled by the pore sizes of polycarbonate membrane templates, whereas peptide nanotubes which were not grown in the membrane pores, were not monodisperse and had to be removed in order to obtain the peptide nanotubes with a uniform diameter. The peptide nanotubes were self-assembled in membrane pores as small as 50 nm in diameter, which was close to the smallest size of the polydisperse peptide nanotubes self-assembled in suspension without templates. Our goal now is to functionalize these monodisperse peptide nanotubes and assemble them into device configurations, such as electronics and sensors, with metal/semiconductor coatings.

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