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Template Synthesis of Nano Test Tubes

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ABSTRACT

We have been investigating potential biomedical and biotechnological applications of template-synthesized nanotubes. One application we envision for these nanotubes is as vehicles for delivery of drugs, DNA, proteins or other biomolecules. The nanotubes that we have prepared previously have been open on both ends – like pipes. For the biomolecule deliver application, it would be best to have nanotubes that are closed on one end and open on the other – nano test tubes. This test tube geometry would allow for convenient filling of the nanotube with the biomolecule of interest, and by applying a cap to the open end, the biomolecule could be kept "bottled-up" inside until it is ready to be delivered. We describe here a simple modification of the template-synthesis method that allows for the preparation of nano test tubes. Prototype silica nano test tubes are described here. We show that the outside diameter of these test tubes is determined by the pore diameter in the template membrane used and that the length of the test tube is determined by the thickness of the template membrane.

Introduction. We have been investigating potential biomedical and biotechnological applications of nanotubes.[1−6] The nanotubes for these studies are prepared by template-synthesis, where tubes of the desired material are deposited along the walls of the pores in a nanopore template membrane (Figure 1). A key advantage of the template method is that nanotubes composed of nearly any desired material can be prepared. Hence, with template synthesis the tube-forming material can be chosen because it fits the biotech application at hand.

One application we envision for such nanotubes is as vehicles for delivery of drugs, DNA, proteins, or other biomolecules.[7−12] Tubular structures are ideally suited for such applications because they are hollow, and with the template method, the inside and outside diameter of the tubes can be controlled at will.[1,2] However, a means for immobilizing the biomolecule within the interior of the nanotubes must be developed. In the past, we have covalently attached the biomolecule to the inside tube walls.[1−3] A much more convenient and general route would be to simply fill the nanotubes with the desired biomolecule and then cap the open ends of the tubes to keep the biomolecule "bottled-up" inside. Immobilization by filling the nanotubes in this way would allow for a larger quantity of the desired biomolecule to be incorporated into the tube. Ideally, the chemistry holding the caps on would be labile, causing the caps to be released in the environment where the biomolecule is to be delivered.

Free-standing membranes were used as the templates in all of our previous investigations of template-synthesized nanotubes.[1−6] Because the pores in a free-standing membrane are open at both membrane faces, the nanotubes obtained were open on both ends, like pipes. It would be much more convenient for bottling a biomolecule within a nanotube if the tubes were open on only one end, like test tubes. This would make it easier to fill the tubes because the biomolecule could not simply flow out of the other open end of the tube during filling. Furthermore, this would make the capping process easier, since only one end of the tube would need to be capped.

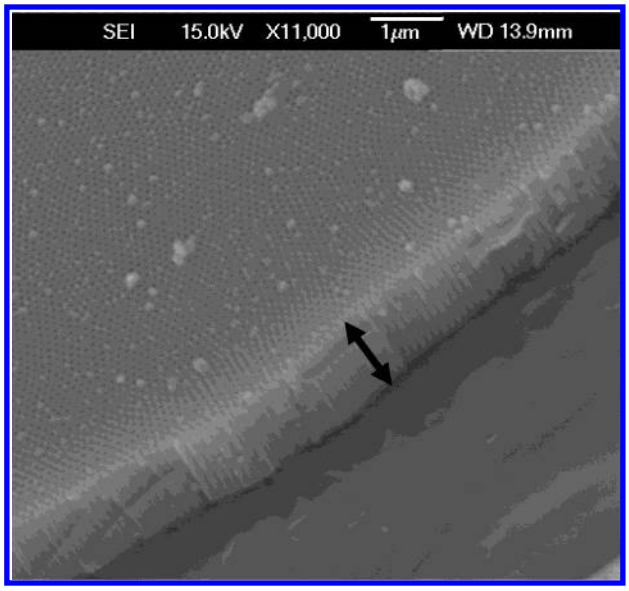

Figure 1. Field emission scanning electron micrograph (FESEM) of the surface and cross section of an alumina template membrane prepared using an anodization voltage of 50 V and an anodization time of 20 min. The cross section is denoted by the double arrow.

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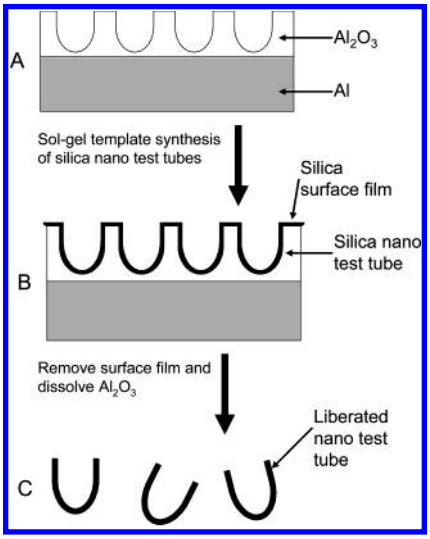

Figure 2. Schematic of the template-synthesis method used to prepare the nano test tubes.

Table 1. Alumina Template Membranes Used to Prepare the Nano Test Tubes

anodization voltage (V)	anodization time (min)	pore diameter (nm)	membrane thickness (μm)
50	10	70	0.6
50	20	70	1.0
70	5	170	0.6

Nanopore alumina membranes prepared by anodic oxidation of the surface of Al metal (Figure 1)[13,14] are one of the workhorse materials for template synthesis of nanotubes.[1−3] It occurred to us that these templates might be ideally suited for preparing nano test tubes because, when still attached to the underlying Al surface, the pores in these membranes are closed at the Al/Al_2O_3 interface (Figure 2A). If the template-synthesis method used deposits the tube-forming material along these closed pore ends (as well as along the pore walls), then nano test tubes would be obtained (Figure 2B). The resulting test tubes could then be liberated and collected by dissolution of the template membrane (Figure 2C). We describe here the application of this approach to make silica nano test tubes. We show that the outside diameter of these test tubes is determined by the pore diameter in the template membrane and that the length of the tube is determined by the thickness of the membrane.

Experimental Section. The nanopore alumina template membranes were prepared in-house by anodic oxidation of high purity aluminum.[14] Two different anodization voltages were used to obtain membranes with pores that were either 70 nm or 170 nm in diameter (Table 1). The thickness of the template membrane was varied by varying the anodization time (Table 1). As indicated in Figure 2, the silica nano test tubes were synthesized with the membrane still attached to the underlying Al surface. However, we needed to measure the template-membrane thickness so that the effect of thickness on nano test tube length could be investigated. Membrane thickness was determined by removing a sample membrane from the aluminum surface[13] and using field-emission scanning electron microscopy (FESEM) to measure the membrane cross section (e.g., Figure 1). The sample membranes used to determine the film thicknesses were prepared using the same anodization times and voltages as the membranes used for template synthesis (Table 1).

A sol−gel method was used to deposit the silica nano test tubes within the pores of these template membranes.[2] The sol precursor solution was prepared by mixing 50 mL of absolute ethanol, 5 mL of tetraethyl orthosilicate and 1 mL of 1 M aqueous HCl. This solution was allowed to hydrolyze for 30 min. The alumina template membrane was then immersed into this sol with sonication for 1 min, and allowed to remain in the sol for an additional 3 min without sonication. The sol-impregnated membrane was then dried in air for 10 min and oven cured overnight at 150 °C. This method yields the desired silica nano test tubes within the pores of the membrane plus a thin surface silica film covering the face of the membrane. This surface film was removed by wiping the membrane surface with a laboratory tissue soaked in ethanol.

The template membrane was then dissolved by overnight immersion into a 25% (wt/wt) solution of H_3PO_4. The liberated test tubes were collected by filtration and rinsed with copious quantities of pH 7.0 phosphate buffer and water. Samples were prepared for transmission electron microscopy (TEM) by resuspending the liberated nano test tubes in ethanol. A TEM grid was immersed into this suspension and the ethanol was allowed to evaporate in air. FESEM was performed using a Hitachi S-4000 microscope. FESEM samples were made conductive by sputtering a thin layer of gold−palladium alloy using a Denton Vacuum Desk II model sputter coater.

We wanted to prove that the silica nano test tubes obtained were, indeed, hollow. We have shown previously that if a polymer solution is applied to the surface of a nanopore template membrane, the solution coats the pore walls, and after evaporation of the solvent, polymeric nanotubes are obtained.[15] We reasoned that if the silica nano test tubes are hollow, they could be used as templates in this way to prepare polymeric nanotubes. A 4% (wt/wt) polystyrene solution was prepared in 50:50 (vol/vol) toluene/dichloromethane. A drop of this solution was placed onto the surface of a silica nano test tube-containing template membrane (i.e., Figure 2B), and the solvent was allowed to evaporate. The template membrane was then dissolved in 1 M NaOH, yielding a film of polystyrene. The surface of this film that was in contact with the membrane was then imaged with the FESEM to see if polystyrene nanotubes (formed in the hollow silica nano test tubes) protrude from this surface. If polystyrene nanotubes are observed, then the nano test tubes must be hollow.

Results and Discussion. In most of our prior work on template synthesized nanotubes, the tubes prepared had very high aspect ratios (nanotube length divided by nanotube diameter). High aspect ratios were not desired here because we wanted nanotubes that resembled test tubes, not long pipes. For this reason, the template membranes used for these studies were thinner than we typically use (Table 1). Figure 3A shows a TEM image of a nano test tube prepared in a template that had 70 nm-diameter pores and was 0.6 μm

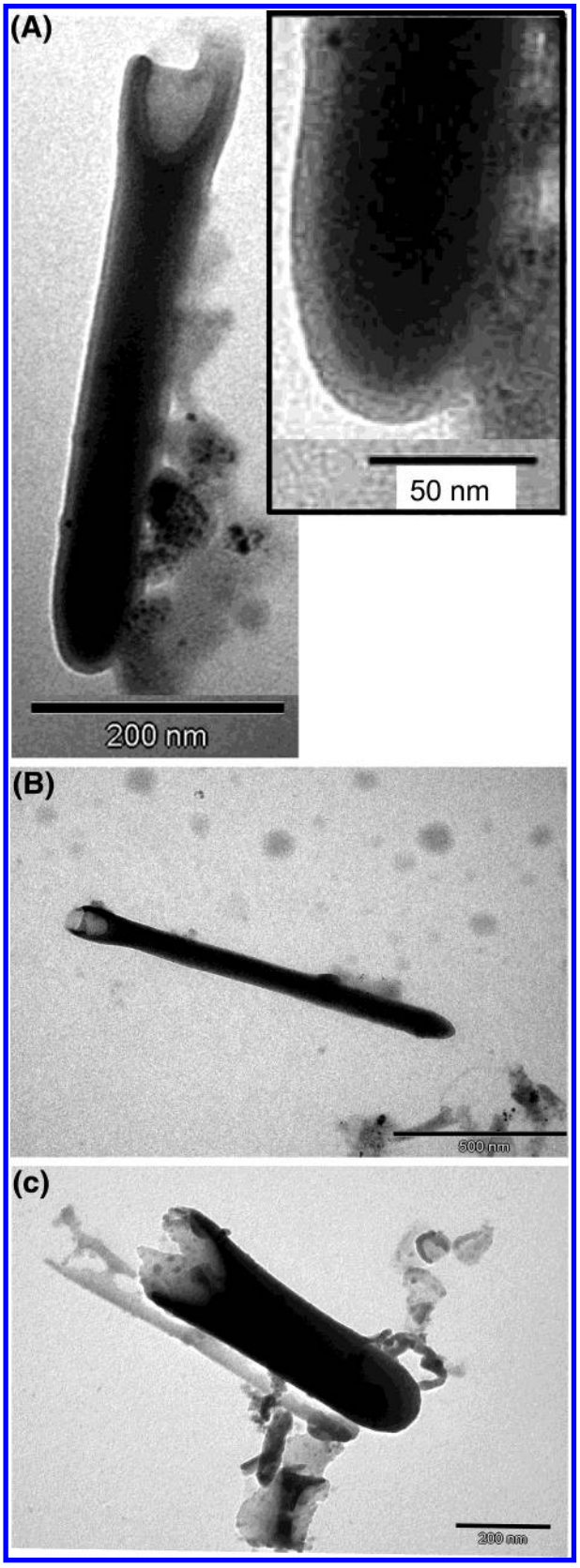

Figure 3. (A) Transmission electron micrograph of a nano test tube prepared in the membrane described in row 1 of Table 1. The inset shows a close up of the closed end of this nano test tube. (B) Transmission electron micrograph of a nano test tube prepared in the membrane described in row 2 of Table 1. (C) Transmission electron micrograph of a nano test tube prepared in the membrane described in row 3 of Table 1.

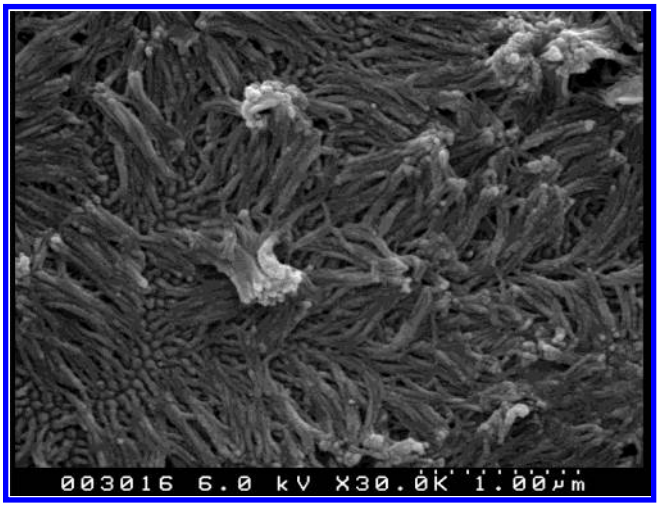

Figure 4. FESEM image of polystyrene nanotubes prepared within the silica nano test tubes.

thick. Both the open end (top of image) and the closed end (bottom of image) of the nano test tube are clearly seen. That the bottom is closed is shown more clearly by the inset in Figure 3A, which is a higher magnification image of the bottom end of this nano test tube. In agreement with the membrane parameters (Table 1), these nano test tubes are 70 ± 14 nm in diameter and ∼600 nm long. Obtaining an accurate value for the length is somewhat problematic because, as indicated in Figure 3, these silica nano test tubes show fracturing at their open ends, where the tube is most fragile.

Figure 3B shows a TEM image of a nano test tube prepared in the membrane that was 1 μm thick (Table 1). Correspondingly, this test tube is ∼1 μm long indicating, as expected, that the length of the test tube can be controlled by varying the thickness of the template. We also wanted to demonstrate that the outside diameter of the nano test tubes could be varied by varying the pore diameter of the template. Figure 3C shows a TEM image of a nano test tube prepared in the membrane with 170 nm diameter pores (Table 1). As would be expected, the diameter of this nano test tube is ∼170 nm, and, again, the length corresponds to the thickness of this template.

The TEM images in Figure 3 do not allow us to unambiguously prove that these silica nano test tubes are hollow. As discussed above, one approach for proving this is to use these test tubes as templates to form nanotubes of a second material. If the test tubes are hollow, then nanotubes of this second material will be obtained. If the test tubes are not hollow, they cannot act as templates, and nanotubes of the second material will not be obtained. Silica nano test tubes prepared in the membrane described in row 2 of Table 1 were used for these studies. Figure 4 shows an FESEM image of the polystyrene nanotubes synthesized within these silica nano test tubes. This image clearly shows that the silica nano test tubes are hollow. Furthermore, the outside diameter of these polystyrene nanotubes (∼35 nm) provides an estimate for the inside diameter of the silica test tubes used. As is typically observed for template-synthesized nanostructures,[15−17] the polystyrene nanotubes aggregate into clumps

after removal of the template membrane. This is undoubtedly due to the advent of capillary forces[18] between the nanotubes upon dissolution of the template membrane.

As shown schematically in Figure 2B, the open end (or top) of the nano test tube corresponds to the surface of the membrane that faced the electrolyte solution during the electrochemical membrane synthesis. As shown in Figures 3 A−C, the open ends of the nano test tubes are always somewhat flared. We have not noticed this flaring in silica nanotubes prepared previously,[2,3] but we have not previously obtained such high resolution images of the tops of such template-synthesized nanotubes. Flaring results because the pores in the thin template membranes used here are correspondingly flared at the surface of the membrane that faced the acidic electrolyte solution during membrane synthesis. This pore flaring results from preferential dissolution of the membrane material at this surface during membrane synthesis.

Conclusions. A sol−gel template synthesis process was used to prepare silica nano test tubes within the pores of alumina template membranes. We have shown that these test tubes are closed on one end, open on the other, and hollow. The length of these test tubes can be controlled at will by varying the thickness of the template membrane used. We have previously shown that the silica nanotubes can be chemically and biochemically functionalized using silane chemistry with commercially available reagents.[2,3] Furthermore, we have shown that different functional groups can be applied to the inner vs outer nanotube surfaces. We are currently using this approach to tag the outer surfaces of the silica nano test tubes with antibodies that promote uptake by endothelial cells. The final challenge to be addressed, if these test tubes are to be used for biomolecule delivery, is to develop procedures for capping and uncapping the open mouth. We will report on this aspect of the research effort soon.

Note Added after ASAP Posting. This paper was originally posted ASAP on 2/5/04. The third author's name has been corrected to be Miguel O. Mota. The corrected version was posted on 2/19/04.

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References

(1) Martin, C. R.; Kohli, P. *Nature Rev. Drug Discov.* **2003**, *2*, 29−37.
(2) Mitchell, D. T.; Lee, S. B.; Trofin, L.; Li, N.; Nevanen, T. K.; Soderlund, H.; Martin, C. R. *J. Am. Chem. Soc.* **2002**, *124*, 11864−11865.
(3) Lee, S. B.; Mitchell, D. T.; Trofin, L.; Nevanen, T. K.; Soderlund, H.; Martin, C. R. *Science* **2002**, *296*, 2198−2200.
(4) Yu, S.; Lee, S. B.; Martin, C. R. *Anal. Chem.* **2003**, *75*, 1239−1244.
(5) Steinle, E. D.; Mitchell, D. T.; Wirtz, M.; Lee, S. B.; Young, V. Y.; Martin, C. R. *Anal. Chem.* **2002**, *74*, 2416−2422.
(6) Yu, S.; Lee, S. B.; Kang, M.; Martin, C. R. *Nano Lett.* **2001**, *1*, 495−498.
(7) Chang, T. M. S.; Prakash, S. *Molecular Biotech.* **2001**, *17*, 249−260.
(8) Allen, C.; Maysinger, D.; Eisenberg, A. *Colloids Surf., B* **1999**, *16*, 3−27.
(9) Ulrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakeshelf, K. M. *Chem. Rev.* **1999**, *99*, 3181−3198.
(10) Soppimath, K. S.; Aminabhavi, T. M.; Kulkarni, A. R.; Rudzinski, W. E. *J. Contol. Release* **2001**, *70*, 1−20.
(11) Thurmond, K. B.; Huang, H.; Clark, C. G.; Kowalewski, T.; Wooley, K. *Colloids Surf.,* **1999**, *16*, 45−54.
(12) Langer, R. *Science* **1990**, *249*, 1527.
(13) Hornyak, G. L.; Patrissi, C. J.; Martin, C. R. *J. Phys. Chem. B* **1997**, *101*, 1548−1555.
(14) Masuda, H. K.; Hasegawa, F.; Ono, S. *J. Electrochem. Soc.* **1997**, *144*, L127.
(15) Cepak, V. M.; Martin, C. R. *Chem. Mater.* **1999**, *11*, 1363−1367.
(16) Li, N.; Martin, C. R. *J. Electrochem. Soc.* **2001**, *148*, A164−A170.
(17) Menon, V. P.; Martin, C. R. *Anal. Chem.* **1995**, *67*, 1920−1928.
(18) Israelachvili, J. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: New York, 1991; pp 330−334.

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