J|A|C|S
COMMUNICATIONS

Published on Web 09/13/2002

Smart Nanotubes for Bioseparations and Biocatalysis

David T. Mitchell,[†] Sang Bok Lee,[†] Lăcrămioara Trofin,[†] Naichao Li,[†] Tarja K. Nevanen,[‡] Hans Söderlund,[‡] and Charles R. Martin*[,†]

Department of Chemistry and Center for Research at the Bio/Nano Interface, University of Florida, Gainesville, Florida 32611-7200 and VTT Biotechnology, PO Box 1500, FIN-02044 VTT Espoo, Finland

Received June 10, 2002

There is enormous current interest in using nanoparticles for biomedical applications including enzyme encapsulation,[1a] DNA transfection, biosensors,[1b] and drug delivery.[1c] Typically, spherical nanoparticles are used for such applications because spherical particles are easy to make. Self-assembling lipid tubules have also been used in biomedical applications although it is difficult to control tubule diameter or length and the lipid tubules must be coated with ceramic or metal to make them rugged enough for biomedical use.[2] We have pioneered a technology, called template synthesis, for preparing monodisperse nanotubes of nearly any size and composed of nearly any material.[3] These nanotubes have a number of attributes that make them potential candidates for biomedical applications. First, nanotubes have inner voids that can be filled with species ranging in size from large proteins to small molecules. In addition, nanotubes have distinct inner and outer surfaces that can be differentially functionalized. The ability to control the dimensions allows for tailoring tube size to fit the biomedical problem at hand. Finally, the ability to make these nanotubes out of nearly any material creates the possibility of making nanotubes with a desired property such as ruggedness or biodegradability.[3c]

In this report we use template-synthesized silica nanotubes (Figure 1A) to demonstrate a number of these concepts. Silica nanotubes are ideal vehicles for such proof-of-concept experiments because they are easy to make, readily suspend in aqueous solution, and because silica surfaces can be derivatized with an enormous variety of chemical functional groups using simple silane chemistry[4] with commercially available reagents. The silica nanotubes were synthesized within the pores of nanopore alumina template membranes (Figure 1B) using a sol−gel method.[4] Templates with pore diameters of either 60 or 200 nm were used for these studies.[5]

We have developed the following simple procedure for applying different functional groups to the inner versus outer surfaces of these nanotubes (see Supporting Information for schematic). While still embedded within the pores of the template membrane, the inner nanotube surfaces are reacted with the first silane. This silane cannot attach to the outer nanotube surfaces because the outer surfaces are in contact with the pore wall and are thus masked. The template is then dissolved[3b] to liberate the nanotubes, which unmasks the outer nanotube surfaces. The nanotubes are then exposed to a second silane to attach this silane to only the outer nanotube surfaces.

To prove this concept, a set of nanotubes was prepared with the green fluorescent silane *N*-(triethoxysilylpropyl)dansylamide attached to their inner surfaces, and the hydrophobic octadecyl silane (C_{18}) to their outer surfaces. These nanotubes were added to a vial containing water and the immiscible organic solvent cyclohexane,

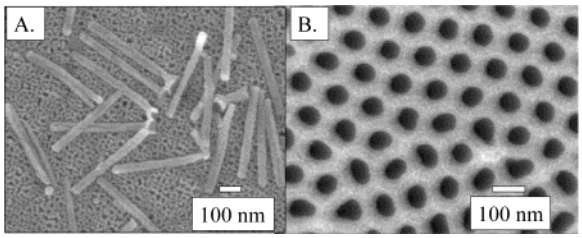

Figure 1. (A) Scanning electron micrograph (SEM) of 60-nm diameter silica nanotubes. The thickness of the alumina template membrane used was 350 nm, which determines the nanotube length. (B) SEM of the surface of a typical alumina template membrane.

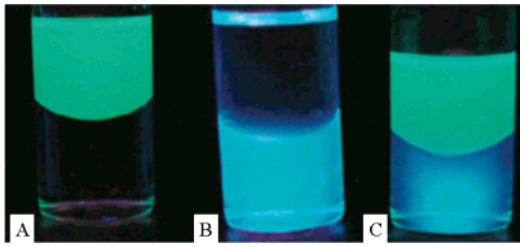

Figure 2. Photographs of vials containing cyclohexane (upper) and water (lower) under UV light excitation after addition of 10 mg of nanotubes with (A) dansylamide on inner and C_{18} on outer surfaces and (B) quinineurethan on inner and no silane on outer surfaces; (C) 10 mg of both A and B nanotubes; 200-nm diameter nanotubes were used.

which were mixed and allowed to separate. Because these nanotubes are hydrophobic on their outer surfaces, they partition into the (upper) cyclohexane phase (Figure 2A). This may be contrasted to nanotubes that were labeled on their inner surfaces with the blue fluorescent silane triethoxysilylpropylquinineurethan, but were not labeled with any silane on their outer surfaces. When the same experiment is done with these nanotubes, the quinineurethane fluorescence is seen only from the aqueous phase (Figure 2B). When both sets of nanotubes are added to the solvent mixture in the same vial, the tubes with the C_{18} outer surface chemistry go to the cyclohexane and the tubes with the silica outer surface chemistry go to the water (Figure 2C).

One application for such differentially functionalized nanotubes is as smart nanophase extractors to remove molecules from solution. Nanotubes with hydrophilic chemistry on their outer surfaces and hydrophobic chemistry on their inner surfaces are ideal for extracting lipophilic molecules from aqueous solution. The hydrophobic 7,8-benzoquinoline (BQ), which has an octanol/water partition coefficient[6] of $10^{3.8}$, was used as a model compound for such nanophase solvent extraction experiments. Five millilgrams of the silica-outer/C_{18}-inner nanotubes were suspended into 5 mL of 1.0×10^{-5} M aqueous BQ. The suspension was stirred for 5 min and then filtered to remove the nanotubes. UV spectroscopy

* To whom correspondence should be addressed. E-mail: crmartin@chem.ufl.edu.
[†] University of Florida.
[‡] VTT Biotechnology.

10.1021/ja027247b CCC: $22.00

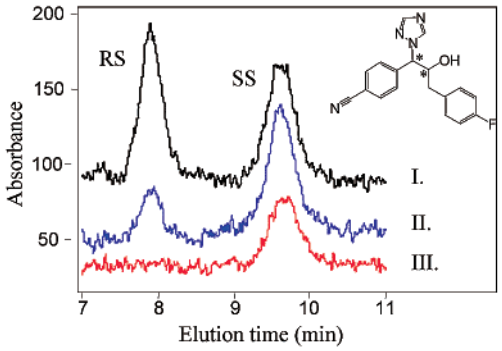

Figure 3. Chiral HPLC chromatograms for racemic mixtures of FTB before (I) and after (II, III) extraction with 18 mg/mL of 200-nm Fab-containing nanotubes. Solutions were 5% dimethyl sulfoxide in sodium phosphate buffer, pH 8.5.

showed that 82% of the BQ was removed from the solution. When a second 5-mg batch of these nanotubes was added, >90% of the BQ was removed from the solution. Control nanotubes that did not contain the hydrophobic C_{18} inner surface chemistry extracted less than 10% of the BQ.

Nanotubes with the C_{18} inside will in principle extract any lipophilic molecule. While this generic extraction ability might be useful for some applications, nanotubes that are molecule-specific would also be useful. We show here that antibody-functionalized nanotubes can provide the ultimate in extraction selectivity—the extraction of one enantiomer of a racemic pair. The antibody was produced against the drug 4-[3-(4-fluorophenyl)-2-hydroxy-1-[1,2,4]-triazol-1-yl-propyl]-benzonitrile (FTB, Figure 3).[7] The antibody used selectively binds the RS enantiomer, and Fab fragments of this antibody were immobilized to both the inner and outer surfaces of the silica nanotubes. This was accomplished by dissolving the template membrane, collecting the nanotubes, and attaching an aldehyde silane to the inner and outer surfaces.[7b] As described previously, this silane reacts via Schiff base chemistry to free amino groups on the protein.[7b]

The Fab-functionalized nanotubes were added to racemic mixtures of the SR and RS enantiomers of FTB. The tubes were then collected by filtration, and the filtrate was assayed for the presence of the two enantiomers using a chiral HPLC method (Figure 3). Chromatogram I is from a solution that was 20 μM in both enantiomers, and chromatogram II was obtained for the same solution after exposure to the Fab-functionalized nanotubes; 75% of the RS enantiomer and none of the SR enantiomer were removed by the nanotubes. When the concentration of the racemic mixture was dropped to 10 μM, all of the RS enantiomer was removed (chromatogram III). Nanotubes containing no Fab did not extract measurable quantities of either enantiomer from the 20 μM solution.

We have also developed a chemistry that allows us to attach the Fab to only the inner surfaces of the nanotubes. While still within the pores of the template membrane, the inner surfaces were treated with aminopropyltrimethoxysilane. The template membrane was then dissolved, and the amino sites on the inner surfaces were coupled to free amino groups on the Fab fragment using the well-known glutaraldehyde coupling reaction. When 18 mg of these interior-only Fab-modified nanotubes were incubated with 1 mL of a 10 μM racemic mixture of the drug, 80% of the RS (and none of the SR) enantiomer was extracted. This corresponds to 0.44 nmol RS enantiomer per mg tubes, whereas almost double that amount, 0.80 nmol/mg, was extracted by the nanotubes with Fab on both their inner and outer surfaces.

The final example concerns the immobilization the enzyme glucose oxidase (GOD) to the silica nanotubes. GOD was immobilized, on both the inside and outside surfaces, via the aldehyde silane route. These GOD nanotubes (60-nm diameter) were dispersed into a solution that was 90 mM in glucose and also contained the components of the standard dianisidine-based assay for GOD activity.[8] A GOD activity of 0.5 ± 0.2 units/mg of nanotubes was obtained. These studies also showed that protein immobilized via the Schiff-base route[7b] is not leached from the nanotubes, in that all GOD activity ceased when the nanotubes were filtered from the solution.

It is important to again emphasize that the template route can be used to prepare nanotubes of nearly any material. We have previously described a procedure for preparing nanotubes composed of the well-known biodegradable polymer poly(lactic acid).[3c] Such biodegradable nanotubes should prove useful for in vivo applications of biomedical nanotube technology.

Acknowledgment. This work was supported by the National Science Foundation, the Office of Naval Research, the UF Engineering Research Center for Particle Science and Technology, and the National Technology Agency, Finland. The FTB was kindly provided by Hormos Medical Ltd.

Supporting Information Available: Procedure for antibody immobilization and schematic for differential funtionalization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Chang, T. M. S.; Prakash, S. *Mol. Biotechnol.* **2001**, *17*, 249. (b) Park, S. A.; Taton, T. A.; Mirkin C. A. *Science* **2002**, *295*, 1503. (c) Langer, R. *Science* **2001**, *293*, 58.

(2) (a) Goldstein, A. S.; Gelb, M. H.; Yager, P. *J. Controlled Release* **2001**, *70*, 125. (b) Spector, M. S.; Selinger, J. V.; Singh, A.; Rodriguez, J. M.; Price, R. R.; Schnur, J. M. *Langmuir* **1998**, *14*, 3493.

(3) (a) Martin, C. R. *Science* **1994**, *266*, 1961. (b) Miller, S. A.; Young, V. Y.; Martin, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 12335. (c) Cepak, V. M.; Martin, C. R. *Chem. Mater.* **1999**, *11*, 1363.

(4) Steinle, E. D.; Mitchell, D. T.; Wirtz, M.; Lee, S. B.; Young, V. Y.; Martin, C. R. *Anal. Chem.* **2002**, *74*, 2416.

(5) (a) The 60-nm pore diameter template was prepared in-house using an electrochemical method described previously.[5b] The 200-nm membrane was obtained commercially (Whatman). (b) Hornyak, G. L.; Patrissi, C. J.; Martin, C. R. *J. Phys. Chem. B* **1997**, *101*, 1548.

(6) De Voogt, P.; Wegener, J. W. M.; Klamer, J. C.; van Zijl, G.; Grovers, H. *Biomed. Environ. Sci.* **1998**, *1*, 194.

(7) (a) Nevanen, T. K.; Soderholm, L.; Kukkonen, K.; Suortti, T.; Teerinen, T.; Linder, M.; Soderlund, H.; Teeri, T. T. *J. Chromatogr. A* **2001**, *925*, 89. (b) Lee, S. B.; Mitchell, D. T.; Trofin, L.; Nevanen, T. K.; Soderlund, H.; Martin, C. R. *Science* **2002**, *296*, 2198.

(8) Bergmeyer, H. U.; Gawehn, K.; Grassl, M. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Acedemic Press: New York, 1974; p 457.

JA027247B