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Corking Nano Test Tubes by Chemical Self-Assembly

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There is tremendous current interest in using nanoparticles to deliver biomolecules and macromolecules (e.g., drugs and DNA) to specific sites in living systems.[1] Release of the biomedical payload from the nanoparticle can be accomplished by chemical[1c] or enzymatic[1d] degradation of the nanoparticle or of the link between the payload and the nanoparticle.[1e] Alternatively, release may be triggered by a pH-[1f] or temperature-induced change in the characteristics of the nanoparticle.[1g] We are exploring an alternative payload-release strategy that builds on our work on template-synthesized nano test tubes.[2] These are hollow nanotubes that are closed on one end and open on the other, and the dimensions can be controlled at will. If these nano test tubes could be filled with a payload and then the open end corked with a chemically labile cap, they might function as a universal delivery vehicle.

The long-range objective would, of course, be to have the bonds holding the corks to the nano test tubes be labile only when a specific chemical signal is encountered (e.g., the lower local pH surrounding certain tumor cells).[3] However, as a first step toward this corked-nano-test-tube strategy, we have shown that template-synthesized silica nano test tubes[2] that are functionalized on the upper rim with amino groups can be spontaneously corked with appropriately sized aldehyde-functionalized latex nanoparticles. Corking involves the Schiff's base reaction to form imine linkages between the test tubes and the nanoparticle corks.

The silica nano test tubes were synthesized via the template method[2] within the pores of nanopore alumina films, prepared in-house by electrochemical oxidation of Al foil.[4,5] The pore diameter was ~80 nm, and the film thickness was ~500 nm. In most of our prior work, the alumina film was detached from the underlying Al surface; for these studies, the film remained attached to the Al (black band at the bottom of Figure 1A).

The sol−gel method used to deposit the silica nano test tubes (Figure 1B) has been described in detail previously.[5,6] Briefly, the Al_2O_3/Al sample was immersed into the sol−gel precursor for 1 min with sonication. The sample was then removed and the surface swabbed with ethanol to remove precursor from the alumina surface. The sample was then dried in air for 10 min and oven cured overnight at 150 °C. This yields silica nanotubes with wall thickness of ~3 nm embedded within the pores of the alumina film.[6] Because the pores in the alumina are closed at their bottoms, the bottoms of the nanotubes are likewise closed (Figure 1B).[2]

While still embedded within the pores of the alumina template, the nano test tubes were reacted with the amino silane 3-aminopropyltrimethoxysilane (APTS).[5] APTS is deposited both on the test tube rim and along the inner walls of the test tube (Figure 1C). After APTS functionalization, the nano test tubes bear cationic surface charge.

The corks used for this work were surfactant-free, polystyrene latex nanoparticles (Interfacial Dynamics). The abbreviations used to denote these nanoparticles, and their average sizes are given in parentheses: (1) anionic sulfate nanoparticles that also contained aldehyde groups (anion-aldehyde, 75 ± 6 nm); (2) cationic amidine nanoparticles that also contained aldehyde groups (cation-aldehyde, 75 ± 6 nm); (3) anionic sulfate nanoparticles, no aldehyde (anion-only, 75 ± 6 nm); (4) cationic amidine nanoparticles, no aldehyde (cation-only, 78 ± 11 nm). The extent of functionalization of each particle is given in the Supporting Information.

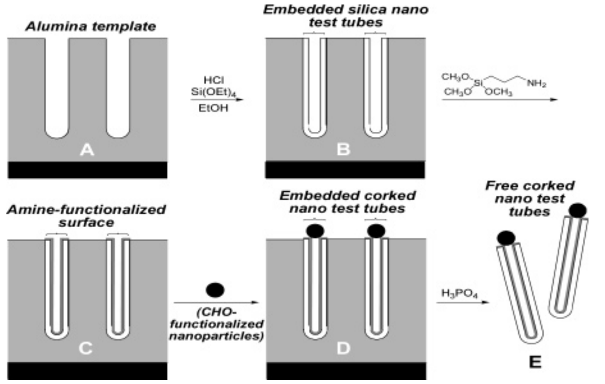

Figure 1. Schematic of the test tube synthesis and corking processes.

The nanoparticles were suspended in aqueous solutions that were 10 mM in NaCl. The nanoparticle concentrations were $\sim 2 \times 10^{12}$ particles per mL (anion-aldehyde, cation-aldehyde, cation-only) and $\sim 3 \times 10^{12}$ particles per mL (anion-only). The sample with the embedded silica nano test tubes was exposed to the nanoparticle solution, with stirring, for 1 h. The sample was then immersed into water and stirred for 2 h to remove any physisorbed particles. The corked nano test tubes were then liberated by dissolving the alumina in 5% H_3PO_4 and collected by vacuum filtration.

As discussed above, prior to thermally processing the sol−gel precursor to convert it to silica, excess precursor was removed from the surface of the template. As a result, after thermal processing, the embedded silica nano test tubes are surrounded by bare alumina, not silica-coated alumina (Figure 1B). This was done because silanes achieve much lower surface coverage on alumina than on silica.[7] Hence, having bare alumina surrounding the amino-functionalized nano test tubes increases the likelihood that the aldehyde-functionalized nanoparticles will deposit within the test tube mouth (Figure 1D) and not on the surrounding surfaces.

The length of the nano test tubes is determined by the thickness of the alumina template (500 nm), and the outside diameter is determined by the diameter of the pores in the template (80 nm). The walls of the nano test tubes are ~3 nm thick,[6] making the inside diameter of the test tubes ~74 nm. Hence, ~75 nm diameter nanoparticle corks were used.

The positively charged APTS-modified nano test tubes were exposed to a suspension of the anion-aldehyde nanoparticles. The molar ratio of nanoparticles to nano test tubes was ~100:1. After exposure, the sample surface was washed extensively with buffer to remove noncovalently bound nanoparticles and imaged with

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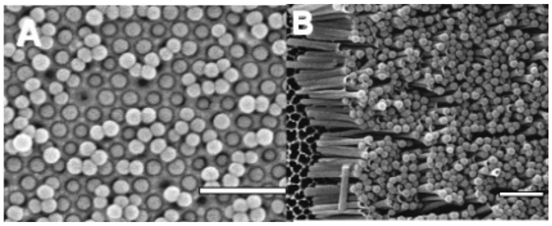

Figure 2. SEM images of anion-aldehyde-corked nano test tubes. (A) Template embedded. (B) Liberated. Scale bar = 500 nm.

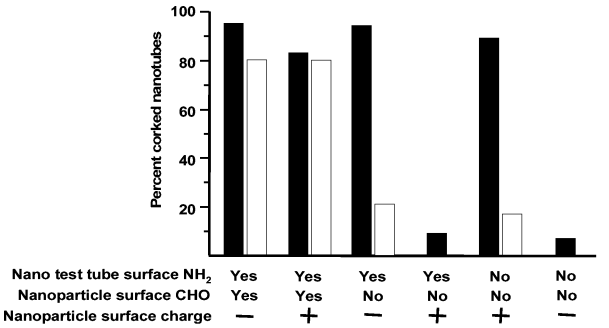

Figure 3. Quantitative analysis of the SEM images. Black bars: data obtained from images of the template-embedded nanotubes. White bars: data obtained from images of the liberated nanotubes.

scanning electron microscopy (SEM) (Figure 2A). Such images showed that the nanoparticles were deposited primarily within the mouths of the nano test tubes, and that 95% of the nano test tubes were corked in this way. Images of the liberated corked test tubes show that 80% of the tubes remain corked (Figures 2B). This strongly suggests that the nanoparticles are attached to the nano test tubes by covalent imine linkages. We carried out several control experiments to confirm this conclusion.

We were particularly concerned about the possibility that the corking observed in Figure 2 was caused by electrostatic interactions between the positively charged nanotubes and the negatively charged anion-aldehyde nanoparticles. To eliminate this possibility, the corking process was repeated with the positively charged cation-aldehyde nanoparticles. Surface SEM images after exposure to these nanoparticles[5] showed that 83% of the nano test tubes were corked. This is only slightly lower than the fraction of corked tubes observed with the anion-aldehyde nanoparticles. After dissolution of the template, the fractions of nanotubes corked with the anion-aldehyde and cation-aldehyde nanoparticles are identical (80%, Figure 3).

To confirm that corking entails formation of covalent imine bonds between the nanoparticles and the nano test tubes, the procedure was repeated with the aldehyde-free anion-only nanoparticles. While the surface SEM images show that 95% of the nanotubes are initially corked, only 20% remained corked after dissolution of the template (Figure 3). These data show that electrostatic corking does occur between the anionic particles and the cationic nanotubes, but unlike the covalently bound corks, these electrostatically bound corks do not survive exposure to the 5% phosphoric acid used to dissolve the alumina template. When the procedure was repeated with the cation-only nanoparticles, very little corking is observed (Figure 3) because the nanotubes and the nanoparticles are both cationic.

Attempts to cork silica nano test tubes whose surfaces were not modified with APTS further emphasized the crucial importance of covalent imine linkages between the tubes and the nanoparticles. When the cation-only nanoparticles were exposed to the APTS-free nanotubes, electrostatic corking occurs, but again most of the corks are lost when the nanotubes are liberated (Figure 3). Electrostatic corking occurs in this case because the silica surface is negatively charged.[8] In contrast, when these negatively charged nanotubes are exposed to the anion-only nanoparticles, very little corking is observed even before dissolution of the alumina template (Figure 3).

We have shown that silica nano test tubes can be covalently corked by chemical self-assembly of nanoparticles to the tubes. The nanoparticle corks remain attached to the mouths of the nano test tubes after liberation from the alumina template. In addition, caps are present only at the mouth and not within the tubes.[5] Applications in drug delivery may require that the outer surfaces of the tubes carry specific moieties (organic functional groups, proteins, nucleic acids, etc.) to direct the nanostructures to their destinations, and our template-based synthesis approach makes it possible to add these modifications after release from the alumina template.[9]

For this proof-of-principle study, we used simple imine linkages to attach the corks to the test tubes. Schiff's bases are thermodynamically unstable in the presence of water; however, the multiple points of contact between the nano test tubes and nanoparticles allow the assembled structure to be metastable under our experimental conditions. Other chemical linkages—either more or less stable—may be more appropriate for other applications, and these are currently under development.

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Supporting Information Available: Details of preparation and functionalization of the nano test tubes. This material is available free of charge via the Internet at http://pubs.acs.org.

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