Novel Bacteria-Templated Sonochemical Route for the in Situ One-Step Synthesis of ZnS Hollow Nanostructures

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Hierarchical hollow nanostructures have attracted a great deal of attention because of their conspicuous physicochemical properties that differ markedly from those of bulk materials and potential for wide-ranging applications especially in optical, electronic, magnetic, and sensing devices ranging from photonic crystals to drug-delivery carriers and nanoreactors.[1] These materials are often prepared by the template synthesis method in which a series of synthetic materials[2] as well as natural organisms[3] have served as templates successfully. But there still exist two main challenges to be overcome: first, synthetic templates should be prepared first via complicated chemical routes whereas some odd and stunning morphologies are currently unattainable which may have striking properties. Therefore, such a template that possesses a variety of well-defined morphologies, unique nanometer-sized dimensions, functionally controllable surfaces, possibility for large-scale production, and inexpensive and environmentally benign qualities is highly appealing. Second, in traditional method removing templates seems indispensable which is multistep and energy-consuming and may cause collapse of structures; thus, developing a straightforward and general method for one-step synthesis of various hollow inorganic replicas with no additional core removal step is of great importance and still a challenge for materials scientists.

In this communication, we report, for the first time, a novel bacteria-templated sonochemical route for the in situ one-step synthesis of ZnS hollow nanostructures at room temperature involving artificial mineralization and microorganism disruption. This facile, novel, and green method has potential as a generic means of the one-step synthesis of various hollow nanostructures of other materials.

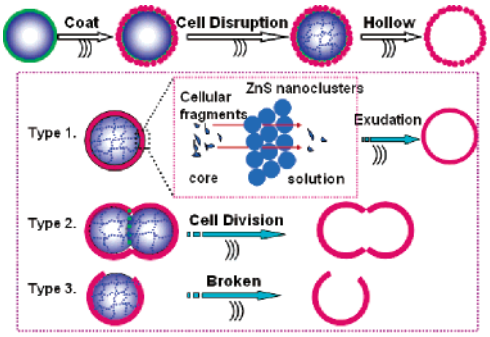

Figure 1. Schematic illustration of the in situ one-step formation of ZnS hollow spheres using *Str. theromophilus* as templates.[2]

Biological templates (bacterium,[4] DNA,[5] virus particles,[6] etc.) have been used to deposit nanoparticles or produce nanostructures. Here, the use of a bacterium as a sacrificial template with the assist of sonochemistry for the one-step synthesis of hollow nanostructures should have significant benefits: Bacteria have evolved a large variety of well-defined stunning morphologies controlled at the micro- or even nanoscopic level, among which cocci, bacillus, vibrios, spirillum, and square bacteria acting as templates can lead to the formation of corresponding three-dimensional hollow nanostructures, some of which are currently unattainable through any other chemical method, and materials of such structures may have striking properties. Moreover, the perfect combination of the two factors (bacteria and sonochemistry) realizes the one-step synthesis of hollow nanostructures, which is a great breakthrough and challenge to the traditional template synthesis process.

We first demonstrate the method with *lactobacillus* as templates because it is familiar and can be easily obtained in large amounts, and we focus on ZnS which can be synthesized by the sonochemical method. Herein, two kinds of *lactobacillus Streptococcus thermophilus* (*Str. theromophilus*) and *Lactobacillus bulgaricus* (*L. bulgaricus*) were used as templates to direct the formation of ZnS hollow spheres and nanotubes, respectively. Figure 1 schematically illustrates the formation process of ZnS hollow spheres. In the first step, ZnS nanoparticles deposit onto the cell surfaces based on the interaction between the functional groups of the cell surfaces and the precursors under sonochemical conditions. Then, ZnS nanoclusters coat cells to form core−shell structures. In the next step, cell disruption takes place

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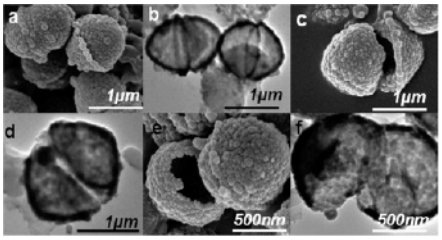

Figure 2. a, b: FESEM and TEM images of ZnS hollow spheres corresponding to type 1 in Figure 1, respectively. c, d: FESEM and TEM images of ZnS hollow spheres corresponding to type 2 in Figure 1, respectively. e, f: FESEM and TEM images of ZnS hollow spheres corresponding to type 3 in Figure 1, respectively.

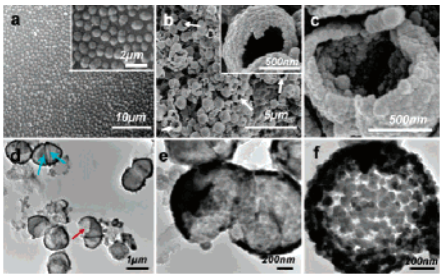

Figure 3. FESEM images of (a) original *Str. theromophilus* template, with the inset at higher magnification, and (b) ZnS hollow spheres at low magnification, with arrows indicating some broken hollow spheres. The inset shows an individual ZnS hollow sphere at high magnification. (c) An individual broken ZnS hollow sphere. TEM images of (d) ZnS hollow spheres at low magnification, (e) ZnS hollow spheres at high magnification, and (f) an individual ZnS hollow sphere.

under ultrasound. Ultrasound has a great effect on the metabolism of the bacterium,[7] which can promote cellular metabolism at low intensity, whereas at high intensity, cells disrupt.[7] In the last step, cellular fragments release from the porous ZnS shells and disperse in the solution, leaving the ZnS shells as hollow spheres. This step can be divided into three types. In type 1, cells disrupt under sonication, and small cellular fragments release through the interstices of the porous shells and disperse in the solution without breaking the integrity of the sphere, leaving the ZnS shells as hollow spheres without any breach. The proposed model can be confirmed in Figure 2a,b. Type 2 illustrates another model of cells just at the stage of cell division. An individual mother cell divides into two daughter cells, and the cellular fragments release from the cross section and disperse in the solution; thus, the hollow twin sphere form. Figure 2c,d coincides with the illustration exactly. Type 3 indicates the formation of some broken hollow spheres. As shown in type 3, cells are coated with ZnS shells with a gap which probably is due to the incomplete coating or to the breakage of original shells, then cellular fragments release, and broken hollow spheres form. Figure 2e,f indicates the broken ZnS hollow spheres.

Figure 3 shows field emission scanning electron microscope (FESEM) and transmission electron microscope (TEM) images of several typical examples using *Str. Theromophilus* as templates. *Str. Theromophilus* (Figure 3a) is approximately

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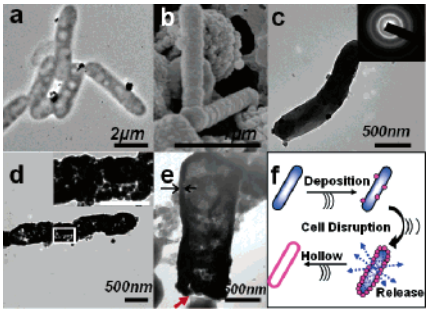


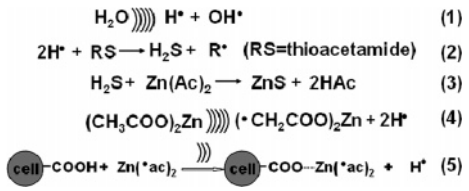
Figure 4. (a) TEM image of the original *L. bulgaricus* template. (b) FESEM image of *L. bulgaricus* coated with a thin layer of ZnS nanoparticles. (c) TEM image of an individual *L. bulgaricus* deposited ZnS nanoparticles without cell disruption; inset shows the corresponding electron diffraction pattern. (d) TEM image of the ZnS hollow tube. The inset is the higher magnification image of the part outlined in the white box showing the crystalline nature of the ZnS nanoparticles. The scale bar of the inset in part d is 500 nm. (e) TEM image of a broken ZnS hollow tube, with the red arrow showing the breach. (f) Schematic illustration of the in situ one-step formation of ZnS hollow nanotubes using *L. bulgaricus* as templates.

spherical with the diameter varying between 0.5 μm and 0.9 μm. After 6 h of sonication, ZnS nanoclusters coat the cell surfaces; simultaneously, the cells disrupt, and the cellular fragments release from the core−shell structures and disperse in the solution, leaving the ZnS shells as hollow spheres (Figure 3b). The inset is a broken sphere at high magnification, showing the conservation of the spherical geometry of the original template. Broken spheres with an apparent cavity demonstrate the hollow nature of the products. The inner part of the hollow sphere (Figure 3c) can be obviously seen, which is made up of ZnS nanoclusters with no cell inclusions left (See Supporting Information). FESEM images of two coat stages reveal the coating nature of ZnS nanoparticles onto the cell surfaces (See Supporting Information). The hollow structure of the as-prepared ZnS spheres is further confirmed by TEM. The pale center together with the dark edge is the evidence for the hollow structure of the microsphere (Figure 3d). During the core removal process in the solution, the hollow spheres faithfully retain the original morphology of the bacterium, and there is no collapse in the spherical symmetry. A typical example is shown by the blue arrow (Figure 3d), indicating the ZnS hollow twin sphere templating of *Str. theromophilus* just at the stage of cell division. The red arrow indicates a broken hollow sphere. The bacteria spheres are almost uniformly coated with ZnS, without any free bare zones. The shells of the hollow spheres are intact with the thickness of about 80 nm (Figure 3e). The TEM image of an individual ZnS hollow sphere at high magnification (Figure 3f) reveals that the ZnS nanoparticles were coated on the cell surfaces as nanoclusters (size∼60-70 nm), and the shells are porous which are helpful for the release of the cellular fragments.

In support of this model, another kind of *lactobacillus L. bulgaricus* which is bacilliform (Figure 4a) is also used as templates. On the average, *L. bulgaricus* has a length of ∼1−5 μm and a width of ∼0.3−0.8 μm. Figure 4b shows *L. bulgaricus* coated with a thin layer of ZnS nanoparticles. After 2 h of sonication, ZnS nanoparticles deposit on the surfaces of *L. bulgaricus* and organic−inorganic tubular composites form because ultrasound has not destroyed the structural integrity of the cells yet (Figure 4c). After

sonication for another 4 h, ZnS hollow nanotubes form followed by the disruption of cells and release of organic residues (Figure 4d). A magnified image of Figure 4d shows that ZnS nanotubes are made up of ZnS nanoclusters with the size of ~60−70 nm. Figure 4e shows a broken ZnS nanotube with a thin layer of about 60−70 nm, and the arrow indicates the breach. The electron diffraction pattern of the hollow ZnS spheres shows diffuse rings, indicating the ZnS spheres are polycrystalline. Figure 4f shows the schematic illustration of the in situ one-step formation of ZnS hollow nanotubes using *L. bulgaricus* as templates which is similar with that of ZnS hollow spheres.

The reactions occurring during sonication which lead to ZnS formation are believed to be[8]



Equations 1−3 represent the main reactions leading to the formation of ZnS nanoclusters. We have replaced $Zn(Ac)_2$ with $ZnCl_2$ and $Zn(NO_3)_2$, but the results were unsuccessful. Thus we believe that the acetate ligand plays an important role in the grafting of zinc onto cell surfaces. Equation 4 represents the formation of $^{\bullet}CH_2COO$ radicals ($^{\bullet}AC$) under sonochemical conditions, which has already been well established.[8,9] The cell wall of *lactobacillus* and many gram-positive bacteria is primarily made up of peptidoglycan (PG), teichoic acid, and teichuronic acid. Additionally, S-layer proteins present as the outermost component of the cell wall are reported to function as templates in the natural mineralization process and are known to bind nanoparticles.[10] These biological components are covered predominantly with carboxyls (R−COOH), phosphomonoesters ($R-OPO_3H_2$), phosphodiesters ($(RO)_2-P(OH)_2$), amines ($R-NH_3^+$), and hydroxyls (R−OH).[11] As the sonochemical treatment ensued, the surfaces of the bacterium have a strong interaction with solute radicals based on the reactivity of these groups and solute radicals (sonochemically formed $Zn(Ac)_2$), producing better adhesion of ZnS nanoparticles. Taking carboxyls as an example, the illustration of the reactivity of carboxyls (R-COOH) and solute radicals is shown in eq 5. So the negatively charged groups as well as ultrasound play significant roles in the mineralization. The zinc acetate implanted into the cell surface then undergoes ligand exchange, with sulfide ions generated according to eq 3, yielding ZnS coated on cells. Once the surface ZnS is formed, this can act as a nucleating site for the further adhesion of ZnS formed in the bulk solution. Additionally, the bacterial cell has a larger surface area than a single ZnS cluster, which means ZnS clusters formed on the surfaces have a larger efficiency and probability to collide with other clusters; as a result, ZnS will cover the bacteria surface.

In conclusion, our results illustrate the potential application of bacteria as templates with the assistance of sonochemistry for the one-step synthesis of ZnS hollow spheres and nanotubes. We have demonstrated the method with cocci and bacillus; our results simultaneously represent an extension of the one-step synthesis of various ZnS hollow nanostructures templating other shapes of bacterium such as vibrios, spirillum, square bacteria, fusiform bacilli, and so forth. Although the current work is focused on ZnS, we believe that a similar approach is applicable to other materials[12] as long as they can be prepared by the sonochemical method and the precursors can have strong interaction with cell surfaces under ultrasound; thus, it is speculated that various hollow nanostructures of some of these materials may be synthesized in one step by this method on the basis of the above premise, which would open up possibilities for extensive study of the physical and chemical properties of these hollow nanostructures and extend their application potentials in catalysis, electrochemistry, biological labeling and diagnostics, sensors, optical devices, and so forth used as articial cells, catalysts, coatings, pigments, and dyes and in protection of light-sensitive components and the controlled release of drugs.[13]

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Supporting Information Available: Experimental details and EDS, FESEM, and XRD data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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